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Original scientific paper

AGRONOMIC, BIOCHEMICAL AND GENETIC ATTRIBUTES OF MAIZE HIGH GRAIN QUALITY ACCESSIONS

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Nutritional quality of maize is low because maize protein is poor in several essential amino acids. The purpose of this research was to analyze agronomic traits and kernel biochemical and physical properties of 16 gene bank accessions which comprise a mini-core collection for grain quality and to identify populations for improving protein quality. Standard ZP341 hybrid was superior for half of agronomic traits tested, especially grain yield, which was higher from 24% to six times. Ten accessions had protein content over 14 % and were further analyzed for amino acid composition and kernel characteristics. Additionally, genetic relationships between the accessions were determined by Simple Sequence Repeats (SSRs) analysis with 30 primers. All accessions showed elevated contents of most essential amino acids. Population L492 with 1.87 and 0.68 g $100g^{-1}$ dry weight had the highest contents of leucine and phenylalanine, respectively, but also higher contents of most other analyzed amino acids ($p<0.05$). Cluster analysis based on SSRs also distinguished L492 by separating it from all other accessions. Compared to ZP341, accessions were significantly inferior in grain weight and dimensions ($p<0.05$), but superior in most hardness parameters ($p<0.05$). Pearson correlations revealed lack of negative correlations between biochemical traits, indicating a possibility for concurrent improvement of several amino acids. The best way of improving protein quality of elite materials is through backcrossing and as populations were chosen according to their good general combining ability (with IoDent, Lancaster and BSSS), they could serve for improvement of elite materials of these genetic origins.

Keywords: breeding, kernel biochemistry, kernel hardness, protein quality.

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INTRODUCTION

Nutritional quality of maize is an important trait as maize is staple food in many undeveloped countries and also one of the major components in livestock feed worldwide. Maize kernel provides 365 kcal 100 g^{-1} and is composed of approximately 73% starch, 10% protein, and 5% oil, with the remainder made up of fiber, vitamins, and micronutrients (JARADAT and GOLDSTEIN, 2013). Proteins, which have vast structural and functional roles within an organism, are of limited quality in maize due to the lack of several essential amino acids, primarily lysine and methionine (FLINT-GARCIA *et al*., 2009). This insufficiency can lead to malnutrition and cause severe diseases in countries where maize is staple food, while in livestock feed, it can negatively influence animal body growth and development.

Variation in physical and biochemical kernel traits is relatively consistent within and among maize hybrids but substantially smaller compared to variation found in landraces and open-pollinated varieties (SEEBAUER *et al*., 2010). Also, proteins are found in much higher concentrations in landraces and old open-pollinated varieties (FUFA *et al*., 2003; SEEBAUER *et al*., 2010). Success in crop improvement through plant breeding depends largely on the existence of genetic variation for the traits of interest in the available gene pool. Thus, maize gene banks in which old landraces as well as synthetic and composite varieties are stored make a promising source of grain quality. Identification of desirable traits and/or genes from unselected or poorly selected genebank materials are used for creating new selection pools and identification of new heterotic patterns in maize (VANČETOVIĆ *et al*., 2015a). Maize Research Institute Zemun Polje gene bank contains 2217 open-pollinated varieties from Western Balkan and 1226 introduced populations (landraces, synthetics and composites). Eighteen populations were recognized for increased grain quality and good universal combining ability with three commercial inbred testers of BSSS, Iodent and Lancaster origin (VANČETOVIĆ *et al*., 2015b) and they comprised a mini core collection for grain quality. The research presented herein deals with agronomic, biochemical and genetic characterization of the mini core collection for grain quality, with emphasis on kernel characteristics, protein and amino acid profiles of these populations. The modes of incorporation of these materials into breeding programs are also discussed.

MATERIALS AND METHODS

Field trials

For the evaluation of agronomic traits, the populations were sown in 2014 in two replications and two plant densities, according to the Randomized Complete Block Design (RCBD), alongside with check hybrid ZP341. A total of 26 agronomic traits, including grain yield (adjusted to 14% moisture) were analyzed. Except for root lodging, significant genetic variability was found for all other traits. Two populations had very low grain yield and they were excluded from further work. The 16 populations finally comprising the mini core collection for grain quality are given in Table 1.

The same experiment was repeated with the 16 populations in 2015 in Zemun Polje, located between geographical coordinates 44°51'N and 20°23'E (altitude: 88 m). In both years elementary plot consisted of two rows with 20 hills 0.4 m apart (plant density - PD I) and 0.3 m

apart (PD II), with row space of 0.75 m. Trials were hand planted, four kernels per hill, and at the 5-7 leaf stage they were thinned to two plants per hill. Plant density I was 66.667 plants ha^{-1} and plant density II 88.889 plants ha^{-1}. Standard agronomic practices for maize production were applied. Harvest was also done by hand.

Table 1. Populations comprising mini core collection for grain quality

	Accession number[1]	Origin	Grain colour	Grain type
1	L 8	Montenegro	yellow	flint
2	L 161	Croatia	yellow	flint
3	L 381	Serbia	white	flint
4	L 449	BIH[2]	yellow	flint
5	L 451	BIH	yellow	flint
6	L 492	BIH	yellow	flint
7	L 499	BIH	white	flint
8	L 626	Serbia	yellow	flint
9	L 933	Slovenia	yellow	flint
10	L 1190	Montenegro	yellow	flint
11	L 1195	Montenegro	orange	flint
12	L 2283	Serbia	yellow	semi dent
13	IP 2314	Russia	yellow	flint
14	IP 5038	Bulgaria	white	flint
15	IP 6429	Italy	yellow	flint
16	IP 7169	Italy	white	flint

[1] - L are landraces from Western Balkan; IP are introduced populations; [2]-BIH - Bosnia and Herzegovina

Analyzed agronomic traits include number of days between emergency and pollination (DP), number of days between emergency and silking (DS), anthesis-silking interval (ASI), number of leafs above the uppermost ear (NL), length of the ear leaf in cm (LL), width of the ear leaf in cm (WL), plant height in cm (PH), height of the uppermost ear in cm (EH), position of the ear on plant given as % (E/PH), % of lodged plants at harvest (L), % of broken plants at harvest (B), number of ears per plant (E/P), % of cob at harvest (C), % of grain moisture at harvest (GM), grain yield in t/ha adjusted to 14% grain moisture (GY), ear length in cm (EL), number of ear rows (NR), number of kernels per row (NK), ear diameter in cm (ED) and cob diameter in cm (CD). Five traits were measured according to the CYMMIT/IBPGR descriptor for maize: tassel type (TT), foliage rating (FR), damage of ear at harvest (DE), ear shape (ES) and regularity of ear rows (RR). Additionally, performance index in comparison to ZP341 (PI), was calculated as:

$$PI = \frac{(GYP \times GMC)}{(GYS \times GMP)} \times 100$$

where GYP is grain yield of the particular population; GMC is the grain moisture of the check hybrid ZP 341 at harvest; GYS is the grain yield of ZP341 and GMP is the grain moisture of the population at harvest.

Biochemical analysis

For biochemical analysis the populations and ZP341 were sawn in 2014 and 2015 in two replications (first one in PD I, and the second one in PD II) according to the Randomized Complete Block Design (RCBD), four rows per each genotype. Selfing was done on this material so that xenia effect would be avoided and 30 selfed ears per population were used for the analyses.

Each accession was represented by 60 kernels (two per ear), divided into two samples of 30 kernels each. Kernels were dried in a controlled oven at 65°C overnight (16-18 hours) and milled in a Cyclotec 1093 lab mill (FOSS Tecator, Sweden) with particle size < 500 μm. The flour was defatted by hexane treatment for 4 hours in a Soxhlet extractor (INKOLAB, Croatia). The protein content (PC) was determined by the standard Kjedahl method using 2200 Kjeltec Auto Distillation Unit (FOSS, Tecator, Sweden), based on nitrogen determination as explained in Vivek et al. (2008). It was estimated from the nitrogen value as: % protein = % nitrogen × 6.25 (conversion factor for maize).

Amino acids were analyzed commercially by the accredited reference SP Laboratory using Ion Exchange Chromatography with electrochemical detector (http://www.splaboratorija.rs). The content values are expressed as g 100 g^{-1} dry weight (DW). Amino acids content of the populations was compared with ZP341 standard hybrid and with ILSI Crop Composition database Version 7 (www.cropcomposition.org), as it was discovered that ZP341, which was selected primarily for its high grain yield under drought conditions, had some ameliorated kernel quality characteristics compared to standard maize.

Kernel physical characteristics

Hundred-kernel weight (100 KW) was measured by weighing 2 × 50 unbroken kernels per sample. Grain test weight (GTW) was estimated using the standard procedure and is given as the average of five measurements in kg m^{-3}. Both 100 KM and GTW were adjusted to 10% moisture. Kernel density (KD) was measured by the ethanol column test method of PAULSEN and HILL (1985) and was expressed as g cm^{3}. Flotation index (FI) was measured by soaking 100 randomly selected kernels into an aqueous sodium nitrate solution with a specific weight of 1.25 g cm^{-3} at 35°C and values were expressed as a percentage of floating kernels. Kernel dimensions were obtained by measuring thickness, width and length of a 100 kernels per sample using a digital micrometer. The hull (KH), endosperm (KE) and germ (KG) were manually dissected after soaking 50 g of kernels for 2 min in 100 mL water. After draining the water, the wet kernels were first manually separated into hull, endosperm and germ, the resulting anatomical parts dried in an oven set at 60°C and weighed in order to calculate relative percentages. The kernel hardness was estimated by modified Stenvert-Pomeranz method, by milling 20 g of kernels in the micro hammer mill (3600 rpm, 2 mm sieve). Results were expressed as milling response (MR) and hard fraction (HF) portion (%). The milling response index presents the time (s) necessary for grain grinding until the top level of the material collected in a glass cylinder (125 × 25 mm) reaches the level of 17 ml.

Genetic Analysis

Genomic DNA was isolated from the kernel bulk according to DOYLE and DOYLE (1987), prepared by pooling an equal amount of flour obtained by grounding 30 kernels per sample. Simple sequence repeat (SSR) analysis was done with 30 primers spanning over the whole genome. Polymerase chain reaction (PCR) was carried out in 25 μl reaction volume containing: 1 × enzyme buffer, 200 μM dNTP, 0.5 μM primers, 1U Taq polymerase and 20 ng DNA template. The following touch-down program (thermocycler Biometra TProfessional Standard 96) was performed: an initial denaturation at 95ºC/5min, followed by 15 cycles each of denaturation at 95ºC/30s, annealing at 63.5ºC/1min (-0.5ºC/cycle) and extension at 72ºC/1min; another 22 cycles of 95ºC/30s, 56ºC/1min and 72ºC/1min with final elongation at 72°C/4min. The amplified fragments were separated by electrophoresis on 8% polyacrylamide gel (Mini Protean Tetra-Cell, BioRad), with 20 bp molecular weight ladder as a marker. After staining with ethidium bromide, gels were photographed under UV light using Biometra BioDocAnalyze Live gel documentation system. SSR profiles were converted into a binary matrix based on the presence (1) or the absence (0) of a specific allele. The pairwise genetic similarity values were calculated on Dice coefficient (DICE, 1945) and UPGMA method was applied for cluster analysis. All marker data analyses were performed using statistical NTSYSpc2 program package.

Statistical methods

For agronomic traits a three-way analysis of variance (ANOVA) of RCBD design was performed (genotype, density and year), alongside with LSD test of differences among genotype means at 0.05 probability level. The same was done for biochemical analysis and kernel traits, except a two-way ANOVA (genotype and year) was performed. Also, Pearson correlations coefficients were calculated for these traits. All the statistical analysis was done in MSTAT-C software.

Among all tested parameters, only those which were highly correlated to the protein content (Table 4) were used for principal component analysis (PCA) and cluster analysis (CA). PLS Toolbox software package v.6.2.1, for MATLAB (R2011a) was used for performing PCA and CA. Analyzed data, before statistical processing, were mean-centered and auto-scaled to unit variance in order to allow the comparison among parameters with different range values.

RESULTS

Field trials

The results of three-way ANOVA for 26 analyzed traits showed that *year* and *genotype* had significant impact on most of the traits, while *replications* and *plant density* were significant for only several traits. *Year* was significant for 21 traits at $p<0.001$ and for ASI at $p<0.05$, but insignificant for WL, NR, ED and CD (data not shown). *Genotype* was insignificant only for L, significant at $p<0.05$ for TT and at $p<0.001$ for all other traits. On the other hand, *replications* were significant for three traits: PI ($p<0.01$), WL and EL ($p<0.05$), while *plant density*

significantly influenced seven traits: ASI *(p<0.05*), TT, E/PH, B, DE, EL (*p<0.01*) and E/P (*p<0.001*).

Fisher's LSD test (data not shown) revealed that hybrid ZP341 was superior for approximately half of the traits, especially for grain yield, which was higher for 24% compared to the next best genotype (L2283) and six times higher compared to the lowest yielding L8. Grain yield of ZP341 was 13.030 t ha^{-1} and grain yield of the populations was in the range from 2.096 to 9.917 t ha^{-1}. However, ZP341 was comparable with the populations for 12 traits – ASI, NL, TT, PH, EH, E/P, L, B, E/P, C, DE and RR.

Biochemical analyses

Protein analysis revealed that PC in six out of 16 quality mini core accessions was below 14 g 100 g^{-1} DW and these accessions were excluded from amino acid, kernel and genetic analyses. Protein content of the remaining 10 accessions was in the range from 14.04 g 100 g^{-1} DW (IP 5038) to 14.91 g 100 g^{-1} DW (L626) with the average content of 14.44 g 100g^{-1} DW.

Two-way ANOVA (data not shown) showed that *year* and *genotype* as well as *year x genotype* interaction had a significant impact on PC. *Year* was significant for eight (proline - Pro, cysteine - Cys and methionine - Met at *p<0.05*, leucine - Leu and phenylalanine - Phe at *p<0.01*, lysine - Lys and valine - Val at *p<0.001*) and *genotype* for five (Phe and aspartic acid - Asp at *p<0.05*, serine - Ser, Leu and Met at *p<0.01*) out of 17 analyzed amino acids. *Year x genotype* interaction was significant only for Asp.

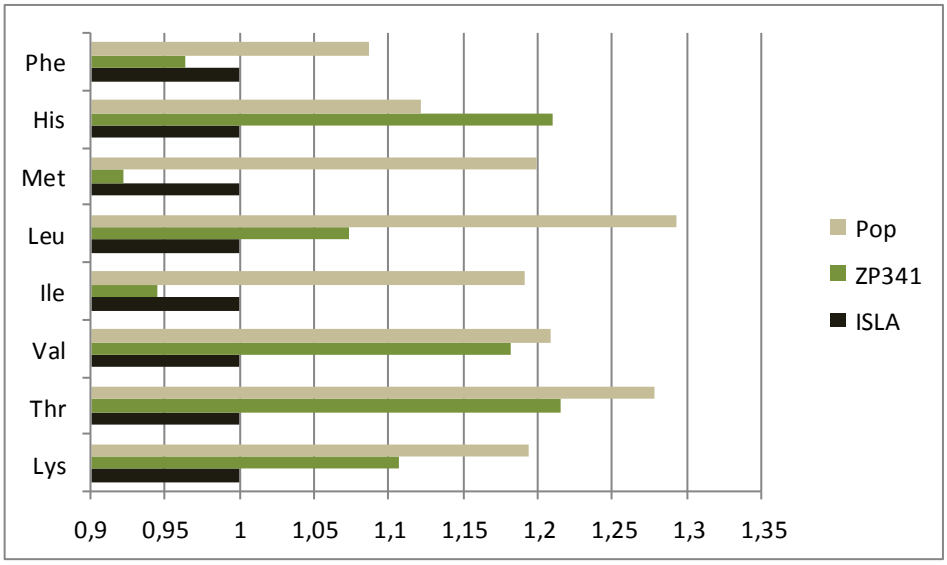

Figure 1. Percentage of difference in average essential AA content between high protein populations, ZP341standard hybrid and values from ILSI Crop Composition database (www.cropcomposition.org). Average contents of amino acids from ILSI are given as 1 (100%).

Table 2. Protein and amino acids content (g 100g^{-1} DW) of mini core collection for grain quality

Trait	Genotype											LSD	Avr.	CV	SD
	L 8	L 161	L 381	L 449	L 492	L 626	L 933	L 1190	IP 2314	IP 5038	ZP341				
PC	14.74ab[1]	14.47abc	14.28abc	14.31abc	14.43abc	14.91a	14.11bc	14.45abc	14.63abc	14.04c	12.55d	*0.64*	14.26	3.07	0.83
Lys[2]	0.38a	0.31a	0.34a	0.39a	0.37a	0.34a	0.34a	0.32a	0.34a	0.32a	0.32a	*0.08*	0.34	14.60	0.07
Ala	0.92ab	0.98a	0.93ab	0.85ab	0.98a	0.91ab	0.98a	0.94b	0.95ab	0.79b	0.81ab	*0.19*	0.92	14.26	0.14
Thr	0.46abc	0.46abc	0.44bc	0.44bc	0.50a	0.46abc	0.46abc	0.46abc	0.49ab	0.46abc	0.44c	*0.05*	0.46	6.60	0.03
Gly	0.40b	0.38b	0.38b	0.41ab	0.43ab	0.40b	0.38b	0.41ab	0.49a	0.37b	0.40b	*0.08*	0.40	14.06	0.06
Val	0.56ab	0.58ab	0.48c	0.54bc	0.62a	0.57ab	0.55abc	0.59ab	0.63a	0.61ab	0.56abc	*0.08*	0.57	10.16	0.08
Ser	0.57bcd	0.59abc	0.60ab	0.57bcd	0.65a	0.60ab	0.53cd	0.58abc	0.61ab	0.58bcd	0.52d	*0.06*	0.58	7.24	0.05
Pro	0.99bc	1.11a	1.04abc	0.94c	1.08ab	0.97bc	0.96c	1.03abc	1.02abc	0.95c	0.96c	*0.11*	1.00	7.67	0.09
Ile	0.39b	0.40b	0.38b	0.37b	0.48ab	0.44ab	0.37b	0.42b	0.59a	0.45ab	0.34b	*0.16*	0.42	25.46	0.12
Leu	1.55bcde	1.79ab	1.69abcd	1.45de	1.87a	1.59bcde	1.47cde	1.69abcd	1.71abc	1.69abcd	1.37e	*0.25*	1.62	10.46	0.24
Met	0.23abc	0.27a	0.25ab	0.22bc	0.27a	0.26ab	0.27a	0.26ab	0.22bc	0.22bc	0.19c	*0.05*	0.24	11.22	0.04
His	0.32ab	0.33a	0.31ab	0.33a	0.34a	0.31ab	0.28b	0.31ab	0.32ab	0.30ab	0.34a	*0.05*	0.32	9.75	0.03
Phe	0.50b	0.54b	0.56b	0.54b	0.68a	0.58b	0.54b	0.57b	0.56b	0.57b	0.50b	*0.09*	0.56	11.22	0.08
Gln	2.30abc	2.34abc	2.23abc	2.06c	2.57ab	2.40abc	2.19bc	2.47abc	2.70a	2.39abc	2.30abc	*0.47*	2.36	13.69	0.38
Asp	0.71bcd	0.66cd	0.63d	0.80ab	0.86a	0.79abc	0.76abcd	0.79abc	0.83a	0.79abc	0.73abcd	*0.13*	0.76	11.48	0.12
Cys	0.16a	0.16a	0.17a	0.18a	0.19a	0.20a	0.19a	0.16a	0.17a	0.16a	0.17a	*0.04*	0.17	13.90	0.03
Tyr	0.32ab	0.30ab	0.25b	0.30ab	0.36ab	0.40a	0.38ab	0.38ab	0.44a	0.37b	0.25b	*0.14*	0.34	27.07	0.09
Arg	0.39abc	0.31c	0.40abc	0.46ab	0.51a	0.41abc	0.38abc	0.38abc	0.38abc	0.43abc	0.35bc	*0.14*	0.40	23.29	0.10

PC–protein content; Lys-lysine; Ala-alanine; Thr-threonine; Gly-glycine; Val-valine; Ser-serine; Pro-proline; Ile-isoleucine; Leu-leucine; Met-methionine; His-histidine; Phe-phenylalanine; Gln-glutamine; Asp-aspartic acid; Cys-cysteine; Tyr-tyrosine; Arg-arginine. [1] - all the values in a row differing in all the letters are statistically different at 0.05 level of significance. [2] – bolded are essential amino acids. LSD – least significant difference at $p<0.05$; Avr. - average value; CV - coefficient of variation (%); SD - standard deviation.

Fisher′s LSD test (Table 2) revealed that all ten analyzed populations had significantly higher PC compared to ZP341. On the contrary, contents of most of the analyzed amino acids were comparable between the populations and ZP341. For seven amino acids (Lys, alanine - Ala, Val, histidine - His, glutamine - Gln, Asp and Cys) no significant differences were found between either populations or populations vs. ZP341. Populations L492 and IP2314 had the highest number of amino acids with significantly higher content compared to ZP341 – L492 was superior in threonine - Thr, Ser, Pro, Leu, Met, Phe and arginine - Arg, and IP2314 in Thr, glycine - Gly, Ser, isoleucine - Ile, Leu and tyrosine - Tyr.

Essential amino acids were compared with both ZP341 and ILSI data for maize grains. The average content of the amino acids (g 100 g^{-1} DW) in ILSI database determined on 8393 maize grain samples are 0.29 for Lys, 0.36 for Thr, 0.47 for Val, 0.36 for Ile, 1.28 for Leu, 0.20 for Met, 0.28 for His and 0.52 for Phe. Percentages of difference for each essential amino acid between ILSI database, ZP341 and populations analyzed are presented in Fig 1. Contents of amino acids from ILSI are given as 1 (100%). It can be noted that populations had the highest content of all essential amino acids, except His which was the highest in ZP341 hybrid.

Kernel physical characteristics

ANOVA (data not shown) revealed that *year* had statistically significant impact on all kernel traits at $p<0.001$ except for MR ($p<0.05$), and it was non-significant for GTW and KL. Similarly, *genotype* was statistically significant at $p<0.001$ for all traits but KEN ($p<0.05$) and non-significant for KE. On the contrary, the effect of *year x genotype* interaction was more diverse - non-significant for KT, KE and KEN, while statistically significant at $p<0.05$ for KDN, KH, MR and HF, at $p<0.01$ for KM and KL, and at $p<0.001$ for GTW, KW and FI.

The results of LSD test (Table 3) revealed that ZP341 was superior to all populations for 100 KW, FI and KL, as well as for KW to all populations except L381. At the same time, this hybrid was inferior in comparison with majority of the populations for GTW and MR, but comparable with half or more populations for the remaining kernel characteristics.

Correlations between biochemical and kernel characteristics

Pearson correlation coefficients between PC and essential amino acids, as well as between essential amino acids themselves are presented in Table 4. All correlations were positive. Protein content was significantly correlated with Lys, Val and Phe at $p<0.05$, as well as with Leu and Met at $p<0.01$. Lysine was the only amino acid not significantly correlated with any of the other essential amino acids. On the contrary, significant correlations were found between Thr and all other essential amino acids ($p<0.001$) except Lys. Similarly, Phe was significantly correlated ($p<0.001$) with all amino acids except Lys and His.

Pearson′s correlation coefficients calculated between biochemical and kernel properties (Table 4) revealed that KW was not significantly correlated with any of the biochemical traits and that His was not significantly correlated with any of the kernel traits. Similar to His, Ile was not significantly correlated with any of the kernel traits except HF ($p<0.01$). The other two kernel dimension traits, KL and KT, showed non-significant correlations with most of the analyzed biochemical traits. However, KL was negatively correlated with PC at $p<0.001$ and Met at $p<0.01$, while KT was positively correlated with Lys ($p<0.01$) and negatively with Leu

Table 3. Kernel physical characteristics of populations from maize mini core collection for grain quality

	100 KW (g)	GTW (kg m^{-3})	FI (%)	Kernel dimensions (cm)			Kernel proportions (%)			Kernel hardness		
				KW	KL	KT	KH	KEN	KE	KDN (g cm^{-3})	MR (s)	HF (%)
L 8	16.31f[1]	74.30ab	48.73bc	0.7175d	0.8350def	0.4125cd	5.878e	81.49abcde	12.63ab	1.26cd	11.99defg	66.79jk
L 161	9.76g	76.03ab	4.89j	0.5725e	0.6550h	0.4050d	7.642abc	82.40abc	9.96b	1.332a	16.58a	73.12a
L 381	29.50b	62.37d	53.88ab	1.0580a	1.0250b	0.4225cd	6.677cde	82.99ab	10.34ab	1.263cd	11.61efgh	67.43ij
L 449	20.00cd	65.90cd	55.76ab	0.7800c	1.0100b	0.4300bcd	6.710cde	82.00abcd	11.29ab	1.243d	10.43h	66.22k
L 492	20.33c	74.49ab	16.89hi	0.7875c	0.9125c	0.4125cd	8.570a	78.59f	12.84a	1.300abc	13.34cd	71.11cde
L 626	17.82def	72.68abc	29.63efg	0.7575cd	0.8200efg	0.4500abcd	6.525cde	80.49bcdef	12.98a	1.263cd	12.29def	70.27def
L 933	17.40ef	69.91bcd	39.03cde	0.7925c	0.7675g	0.4875a	6.020e	83.25a	10.73ab	1.267bcd	12.53cdef	69.97efg
L 1190	20.42c	78.12a	22.88gh	0.7925c	0.8650cde	0.4550abc	7.765abc	81.53abcde	10.71ab	1.290abc	12.16def	70.56def
IP 2314	15.39f	74.16ab	34.41def	0.7600cd	0.7825fg	0.4300bcd	8.575a	80.14cdef	11.29ab	1.277bcd	15.19ab	72.83ab
IP 5038	19.60cde	64.34d	26.05fgh	0.7775c	0.8925cd	0.4550abc	8.682a	79.52def	11.80ab	1.295abc	13.90bc	72.11abc
ZP341	34.84a	62.01d	58.82a	0.9050b	1.2430a	0.4725ab	8.015ab	79.83cdef	12.15ab	1.26cd	10.57gh	63.78l
$LSD_{0.05}$	2.477	8.176	10.520	0.0465	0.06576	0.0465	1.279	2.597	2.810	0.04549	1.432	1.155
Average	20.125	70.391	34.721	0.791	0.892	0.439	7.383	80.953	11.666	1.278	12.604	69.728
CV(%)	8.37	7.90	21.07	3.20	4.68	6.77	12.05	2.23	16.74	1.88	7.90	1.15
SD	6.78	7.85	22.62	0.12	0.15	0.05	1.75	3.52	2.43	0.04	1.86	2.68

100KW – 100 kernel weight; GTW-grain test weight; FI-flotation index; KW-kernel width; KL-kernel length; KT-kernel thickness; KH-kernel hull; KEN-kernel endosperm; KE-kernel embryo; KDN-kernel density; MR-milling response; HF-hard fraction portion. [1] - all the values in a column differing in all the letters are statistically different at 0.05 level of significance. LSD-least significant difference at $p<0.05$; CV - coefficient of variation; SD - standard deviation.

(*p<0.01*). Both 100KW and GTW showed significant correlations with PC and Met, but with different trends: 100KW was negatively (*p<0.001* and *p<0.05*, respectively) and GTW positively (*p<0.05*) correlated with these two traits. Additionally, GTW was positively correlated with Thr at *p<0.05*. Considering kernel proportion traits, significant correlations were found with Lys, Thr, Val and Phe. The highest number of significant correlations was found between HF and biochemical characteristics – this kernel hardness trait was correlated with PC and all essential amino acids except Lys and His.

Table 4. Pearson correlation coefficients between kernel biochemical and physical traits

	PC	Lys	Thr	Val	Ile	Leu	Met	His	Phe
Lys	0.372*****								
Thr	ns	ns							
Val	0.331*	ns	0.625*******						
Ile	ns	ns	0.556*******	0.726***					
Leu	0.448**	ns	0.592*******	0.672***	0.587***				
Met	0.404**	ns	0.506*******	0.330*	ns	0.646***			
His	ns	ns	0.646*******	0.421**	ns	ns	ns		
Phe	0.337*	ns	0.596*******	0.652***	0.523***	0.772***	0.566***	ns	
100KM	-0.550***	ns	ns	ns	ns	ns	-0.406*	ns	ns
GTW	0.314*	ns	0.347*	ns	ns	ns	0.330*	ns	ns
FI	ns	0.447**	ns	ns	ns	-0.578**	ns	ns	ns
KD	ns	-0.422**	ns	ns	ns	0.584**	ns	ns	ns
KW	ns	ns	ns	ns	ns	ns	ns	ns	ns
KL	-0.495***	ns	ns	ns	ns	ns	-0.425**	ns	ns
KT	ns	0.429**	ns	ns	ns	-0.507**	ns	ns	ns
KH	ns	0.468**	0.395**	0.478**	ns	ns	ns	ns	0.460**
KEN	ns	0.610***	-0.331*	0.499**	ns	ns	ns	ns	-0.445**
KG	ns	0.548**	ns	0.381*	ns	ns	ns	ns	0.316*
MR	0.336*	ns	ns	0.138*	ns	0.506**	0.393**	ns	ns
HF	0.524***	ns	0.422**	0.472**	0.492**	0.637**	0.479**	ns	0.395**

PC–protein content; Lys-lysine; Thr-threonine; Val-valine; Ile-isoleucine; Leu-leucine; Met-methionine; His-histidine; Phe-phenylalanine; 100KW – 100 kernel weight;

GTW-grain test weight; FI-flotation index; KW-kernel width; KL-kernel length; KT-kernel thickness; KH-kernel hull; KEN-kernel endosperm; KE-kernel embryo;

KDN-kernel density; MR-milling response; HF-hard fraction portion. *,**,*** - statistically significant at 0.05, 0.01 and 0.001 level, respectively.

Principal Component Analysis

Performed principal component analysis resulted in four-component model which explain 90.91 % of the total variance. PC_1 component explained the 54.48 %, while the PC_2 explained the 15.16 % of the overall data variance. For these PCs, mutual projections of the factor scores and loadings are represented in Fig. 2 a and b, respectively.

Physical kernel parameters (i.e. KL and 100KW) separated standard hybrid ZP341 from all the tested populations (Fig. 2a). PCA score showed that Lys content influenced grouping of samples L8, L381 and L449, while the content of amino acids Leu and Val as well as the hardness parameters HF and MR affected the formation of a group of samples L1190, L161,

IP2314 and IP503. According to PC, Met, Phe and HF formed the group of samples L933, L626 and L492.

Dendrogram presented in Fig. 3 mainly confirmed the results from PCA. According to the dendrogram, two clusters of all tested populations, at the difference of approximately 6, were formed. The first cluster consisted of populations L8, L449, L381 and hybrid ZP341, which

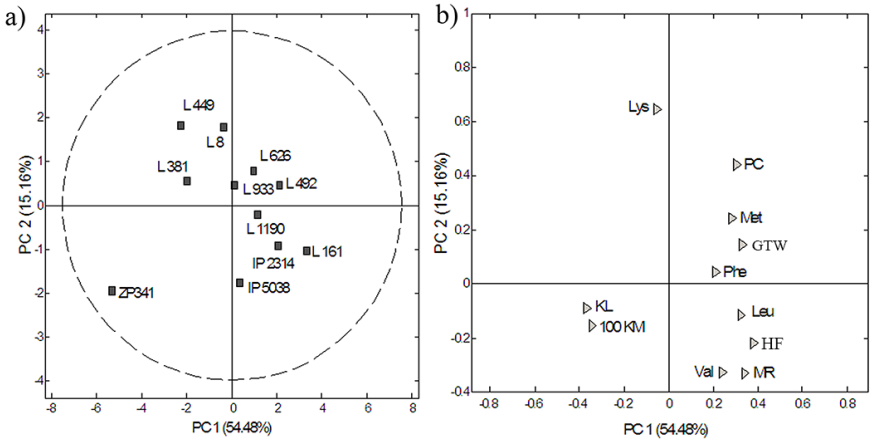

Figure 2. PCA score (a) and loading plot (b) for PC_1 and PC_2 components. PC–protein content; Lys-lysine; ; Val-valine; Leu-leucine; Met-methionine; Phe-phenylalanine; 100 KM – 100 kernel weight; KL-kernel length; MR-milling response; HF-hard fraction portion; GTW – grain test weight.

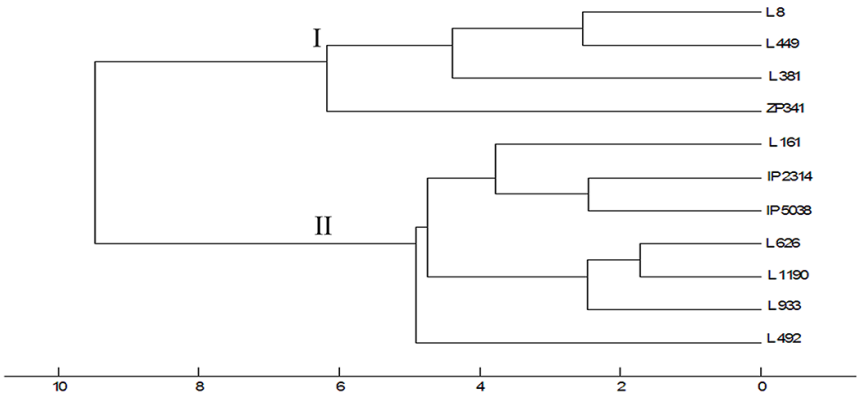

Figure 3. Classification of high protein populations based on significantly correlated grain biochemical and physical characteristics.

Genetic analysis

The analysis of SSR data using Dice coefficient revealed genetic similarities (data not shown) in the range from 0.482 (between ZP 341 and L1190, L626, L161) to 0.756 (between L449 and L933). All the other genetic similarities were in the range from 0.487 (between L1190 and L161) to 0.741 (between L381 and L933). Cluster analysis based on the genetic similarities resulted in a dendrogram presented in Fig. 4. Analyzed populations were divided into two clusters, with adjacent L492 and hybrid ZP 341. Cluster I comprised of four local populations and IP5038, while cluster II comprised of the three local populations and IP2314.

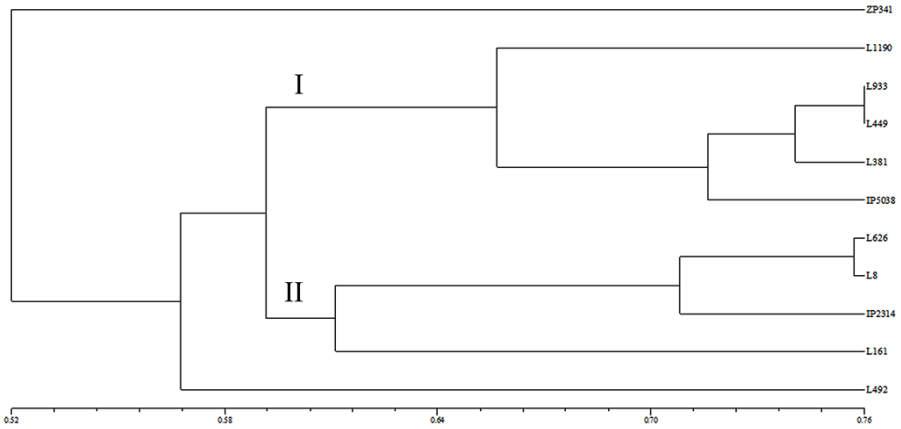

Figure 4. Genetic classification of high protein populations based on SSR marker analysis using Dice coefficient and UPGMA clustering method.

DISCUSSION

The importance of discovering new sources of maize kernel nutritional quality is supported by the expectation that maize consumption will increase by 16 % until 2027, mainly due to the growing human populations and expanding livestock production in developing countries (http://www.fao.org/docrep/i9166e/i9166e_Chapter3_Cereals.pdf). In this context, searching for new sources of protein quality should be considered as one of the main goals, because maize has suboptimal levels of several essential amino acids (GALILI and AMIR, 2012) and thus low biological value.

Biochemical analysis of the mini core collection for grain quality revealed high protein content in all the accessions. Typical kernel composition values for the commodity yellow dent corn on a dry matter basis are 71.7% starch, 9.5% protein, 4.3% oil, 1.4% ash, and 2.6% sugar (WATSON, 2003). Although PC of six accessions was also high, in the range from 12.69 to 13.90 g 100 g^{-1} DW, they were discarded from further analysis as we set the limit to 14.00 g 100 g^{-1} DW protein for amino-acid profile determination. This was done because protein content this high is not common even in the landraces, in which it rarely exceeds 14g 100g^{-1}DW (BERARDO

et al., 2009; FLINT-GARCIA *et al*., 2009; NANKAR *et al*., 2016; IGNJATOVIC-MICIC *et al*., 2015). However, PC is not sufficient by itself and protein quality (essential amino acid composition) determines the nutritional and biological value of a maize genotype.

The most important limiting amino acids in human nutrition and monogastric animal feeds are lysine, tryptophan, methionine and threonine and their deficiency can cause many diseases and body growth limitations (UFAZ and GALILI, 2008; NASR, 2011; CHANG *et al*., 2000; GALILI and AMIR, 2012). Moreover, lysine is the first limiting amino acid and much effort has been put into developing high lysine maize genotypes. The result was Quality Protein Maize (QPM), high lysine/tryptophan maize, created in CIMMYT by using the natural *opaque2* mutation (VIVEK *et al*., 2008). Lysine content in the analyzed accessions was higher than in standard maize kernels for approximately 26% (MBUYA *et al*., 2011; KIL *et al*., 2014) and at the level of blue maize accessions from Brazil (NANKAR *et al*., 2016) and lines developed through Germplasm Enhancement of Maize (GEM) project (SCOTT and BLANCO, 2009). Besides high lysine content, high tryptophan content can also be assumed, as these two amino acids are highly correlated with lysine to tryptophan ratio 3:1 (NURIT *et al*., 2009).

In avian species, methionine is classified as the first limiting amino acid because there is a strong requirement for it to support feather growth and protein synthesis. Similar to QPM, high-methionine inbred lines were developed by using *dzr1* mutation that results in overproduction of a methionine-rich zein (PHILLIPS *et al*., 2008). Methionine content of the analyzed accessions was lower compared to blue maize accessions and GEM lines for approximately 20% (NANKAR *et al*., 2016; SCOTT and BLANCO, 2009), similar to the standard maize lines described in MBUYA *et al*. (2011) but higher for approximately 38 % than in standard maize analyzed in KIL *et al*. (2014). On the contrary, Thr content was higher in all accessions compared to standard lines (MBUYA *et al*. 2011; KIL *et al*., 2014) for approximately 30 and 40%, respectively. Considering that the average contents of the analyzed essential amino acids were higher in all accessions compared to ZP341 hybrid (except His which was the highest in ZP341) and average values given in ILSI Crop Composition Database, as well as literature data for standard maize, it could be concluded that the mini core collection for grain quality comprises accessions with elevated contents of most essential amino acids.

Physically, size and shape of the kernel along with its mass, density, resistance to milling and compression define the hardness of maize kernel (FOX and MANLEY, 2009). Hard kernels produce higher quality of dry milled products (flours, grits, breakfast and snack foods), while soft kernels are more suitable for wet milling due to shorter steeping time and easier separation of starch and protein (GUSTIN *et al*., 2013). The analyzed MRIZP mini core grain quality accessions are potential sources of target alleles for breeding genotypes with improved protein quality and their kernel parameters are to be considered in that context. Kernels with 100KW greater than 32 g and FI less than 20 % are desirable in food processing (SERNA-SALDÍVAR, 2010). None of the analyzed accessions met the criterion for 100KW. However, great variability was found for FI among the analyzed accessions. One of the hardness classifications based on FI indicates that very hard kernels have values between 0 and 12%, hard kernels between 13 and 37%, intermediary hard between 38 and 62%, and soft kernels over 63%. Based on this definition, none of the accessions had soft kernels, four had hard kernels but with FI over

20% and another four had intermediary hard kernels. Only two accessions had desirable FI values for food processing, with L161 having very hard and L492 hard kernels.

Kernels dimensions of the analyzed accessions were significantly smaller in comparison with ZP341 and the difference in size was most prominent for KL. On the contrary, kernel hardness parameters of most accessions were significantly higher (MR and HF) or at the level (KD) of ZP341. The average value of MR was lower and HF higher compared with 20 commercial hybrids analyzed in RADOSAVLJEVIC *et al*. (2000). In IGNJATOVIC-MICIC *et al*. (2015), MR of 13 high oil accessions was higher, but HF was the same as in our research. The results for MR and HE also coincide with results presented for high oil accessions and Top-Cross blends in VANCETOVIC *et al*. (2017). Considering KD, the values found for the accessions were in concurrence with values detected for maize hybrids in ARMSTRONG and TALLADA (2012). Additionally, GTW, which is also an indicator of kernel hardness, was at the level of or higher than in ZP341. Among the analyzed accessions, L161 was superior to other ones and to ZP341, with the highest values of all three kernel hardness parameters. Based on all analyzed kernel physical parameters, it could be inferred that accessions from mini core for protein quality have small and hard kernels.

Positive correlations are important for parallel improvement of different traits (SCOTT and BLANCO, 2009) and the lack of negative correlations between the analyzed biochemical components suggest that it should be possible to improve content of different amino acids simultaneously. However, negative correlations between some biochemical components and kernel physical properties could hinder the breeding progress of kernel protein quality. Consequently, the purpose of improvement has to be well established. For example, significant negative correlations between Met and KM and KL imply that genotypes with higher Met content would have smaller seed sizes, but strong positive correlations between this amino acid and kernel hardness traits (HM, MR and HE) indicate desirable characteristics for dry milling.

Population L492 displayed a distinguishing amino acid profile which was supported by the cluster analyses. This yellow flint landrace originating from Bosnia and Herzegovina excelled its amino acid composition as it had pronouncedly the highest contents of Leu and Phe, but also significantly higher contents of most of the other analyzed amino acids. Cluster analysis based on Dice genetic similarities separated this population from all other populations. Similarly, although a high degree of overlapping was observed in PCA, cluster analysis based on correlated biochemical and kernel physical properties positioned L492 as an adjoined unit to the second sub-cluster.

It has been shown that developing maize cultivars with high grain nutritional quality while maintaining high grain yield, although difficult, is a feasible task (MITTELMANN *et al*., 2003; JARADAT and GOLDSTEIN, 2013). On the other hand, finding new sources of grain quality in germplasm collections can result in creating new selection pools as well as identification of new heterotic patterns in maize. Considering material searched herein, the best possible way of its incorporation into commercial breeding program for improved protein and grain quality is through backcrossing with elite materials. Two backcrosses would be desirable because accessions had poor agronomic values. Since populations from this research were chosen according to their good general combining ability (with IoDent, Lancaster and BSSS bacground), they could serve for the improvement of elite materials of these genetic origins.

CONCLUSION

Biochemical analysis of the mini core collection for improved grain quality in maize identified ten populations with protein content over 14 % and elevated contents of essential amino acids. Analysis of kernel physical characteristics showed that all populations had small and hard kernels. The lack of negative correlations between the analyzed biochemical components was found, but also several significant negative correlations between the biochemical traits and kernel characteristics. According to the results, different populations can be used for simultaneous improvement of contents of different amino acids. Moreover, several populations that displayed good kernel characteristics have the potential for developing genotypes with upgraded amino acids aimed for dry milling. The identified biochemical profiles reflect the profusion of amino acids retained in genebank accessions and highlight them as notable sources for breeding maize with improved protein quality.

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REFERENCES

ARMSTRONG, P.R., J.G., TALLADA (2012): Prediction of kernel density of corn using single-kernel Near Infrared spectroscopy. Appl. Eng. Agric, *28*: 569-574.

BERARDO, N., G., MAZZINELLI, P., VALOTI, P., LAGANA, R., REDAELLI (2009): Characterization of maize germplasm for the chemical composition of the grain. J. Agric. Food Chem., *57*: 2378-2384.

CHANG, W.H., J.H., LEE, K.N., HEO, K., PAIK, I.K., HAN (2000): Optimal threonine: lysine ratio for growing pigs of different sexes. Asian-Australas J. Anim. Sci., *13*: 1731-1737.

DICE, L.R. (1945): Measures of the amount of ecologic association between species. Ecology, *26*: 297-302.

DOYLE, J.J., J.L., DOYLE (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, *19*: 11-15.

FLINT-GARCIA, S.A., A.L., BODNAR, P., SCOTT (2009): Wide variability in kernel composition, seed characteristics, and zein profiles among diverse maize inbreds, landraces, and teosinte. TAG, *119*: 1129-1142.

FOX, G., M., MANLEY (2009): Hardness methods for testing maize kernels. J. Agric. Food Chem., *57*: 5647-5657.

FUFA, H., G., AKALU, A., WONDIMU, S., TAFFESSE, T., GEBRE, K., SCHLOSSER, H., NOETZOLD, T., HENLE (2003): Assessment of protein nutritional quality and effects of traditional processes: A comparison between Ethiopian quality protein maize and five Ethiopian adapted normal maize cultivars. Nahrung/Food, *47*: 269-273.

GALILI, G., R., AMIR (2012): Fortifying plants with the essential amino acids lysine and methionine to improve nutritional quality. Plant Biotechnol. J., 10.111/pbi.12025.

GUSTIN, J.L., S., JACKSON, C., WILLIAMS, A., PATEL, P., ARMSTRONG, G.F., PETER, A.M., SETTLES (2013): Analysis of Maize (*Zea mays*) Kernel Density and Volume Using Micro computed Tomography and Single-Kernel Near-Infrared Spectroscopy. J. Agric. Food Chem., *61*: 10872-10880.

IGNJATOVIC-MICIC, D., J., VANCETOVIC, D., TRBOVIC, Z., DUMANOVIC, M., KOSTADINOVIC, S., BOZINOVIC (2015): Grain Nutrient Composition of Maize (*Zea mays* L.) Drought-Tolerant Populations. J. Agric. Food Chem., *63*: 1251-1260.

JARADAT, A.A., W., GOLDSTEIN (2013): Diversity of Maize Kernels from a Breeding Program for Protein Quality: I. Physical, Biochemical, Nutrient, and Color Traits. Crop Sci., *53*: 956-976.

KIL, D.Y., C.S., PARKB, A.R., SONB, S.Y., JIC, B.G., KIMB (2014): Digestibility of crude protein and amino acids in corn grains from different origins for pigs. Anim. Feed Sci. Techn., *196*: 68-75.

MBUYA, K., K.K., NKONGOLO, A., KALONJI-MBUYI (2011): Nutritional Analysis of Quality Protein Maize Varieties Selected for Agronomic Characteristics in a Breeding Program. Int. J. Plant Breeding Genet., *5*: 317-327.

MITTELMANN, A., J.B.,DE MIRANDA FILHO,; G.J., MELLO MONTEIRO DE LIMA, C. HARA-KLEIN, R.T., TANAKA (2003): Potential of the ESA23B maize population for protein and oil content improvement. Sci. Agri., *60*: 319-327.

NANKAR, A., L., GRANT, P., SCOTT, R.C., PRATT (2016): Agronomic and Kernel Compositional Traits of Blue Maize Landraces from the Southwestern United States. Crop Sci., *56*: 2663-2674.

NASR, J. (2011): Effect of different levels of amino acids on carcass composition and yield in broilers. Animal Production Science, *51*:1123-1126.

NURIT, E., A., TIESSEN, K., PIXLEY, N., PALACIOS-ROJAS (2009): A reliable and inexpensive colorimetric method for determining protein-bound tryptophan in maize kernels. J. Agric. Food Chem., *57*: 7233-7238.

PHILLIPS, R.L., J., SURESH, M., OLSEN, T., KRONE (2008): Registration of high-methionine versions of maize inbreds A632, B73, and Mo17. Journal of Plant Registration, *2*: 243-245.

RADOSAVLJEVIC, M., V., BEKRIC, I., BOZOVIC, J., JAKOVLJEVIC (2000): Physical and chemical properties of various corn genotypes as a criterion of technological quality. Genetika, *32*:319-329.

SCOTT, M.P., M., BLANCO (2009): Evaluation of the grain methionine, lysine and tryptophan contents of maize (*Zea mays* L.) germplasm in the Germplasm Enhancement of Maize Project. Plant Genet. Resour., *7*: 237-243.

SEEBAUER, J.R., G.W., SINGLETARY, P.M., KRUMPELMAN, M.L., RUFFO, F.E., BELOW (2010): Relationship of source and sink in determining kernel composition of maize. J. Exp. Bot., *61*: 511–519.

SERNA-SALDÍVAR, S.O. (2010): Cereal grains: properties, processing, and nutritional attributes. Series Food Preservation Technology; Gustavo, V. Barbosa-Canovas, Eds.; CRC Press, Boca Raton, FL, USA.

UFAZ, S., G., GALILI (2008): Improving the Content of Essential Amino Acids in Crop Plants: Goals and Opportunities. Plant Physiol., *147*: 954-961.

VANČETOVIĆ, J., S., BOŽINOVIĆ, D., IGNJATOVIĆ-MICIĆ, N., DELIĆ, N., KRAVIĆ, A., NIKOLIĆ (2015a): A diallel cross among drought tolerant maize populations. Euphytica, *205*: 1-16.

VANČETOVIĆ, J., D., IGNJATOVIĆ-MICIĆ, S., BOŽINOVIĆ, N., DELIĆ (2015b): Maize core collection for increased grain quality. Maydica, *60*: M32.

VANCETOVIC, J., D., IGNJATOVIC-MICIC, D., TRBOVIC, N., DELIC, S., BOZINOVIC, A., NIKOLIC, M., KOSTADINOVIC (2017): Biochemical and physical kernel properties of a standard maize hybrid in different TopCrossTM Blends. Sci. Agri., *74*: 461-468.

VIVEK, B.S., A.F., KRIVANEK, N., PALACIOS-ROJAS, S., TWUMASI-AFRIYIE, A.O., DIALLO (2008): Breeding Quality Protein Maize (QPM): Protocols for Developing QPM Cultivars. Mexico, D.F.: CIMMYT.

WATSON, S.A. (2003): Description, development, structure and composition of the corn kernel. In: Corn: Chemistry and Technology, 2nd edition; White, J., Johnson, L., Eds.; American Association of Cereal Chemists: St. Paul, MN, USA, pp 69-101.

AGRONOMSKA, BIOHEMIJSKA I GENETIČKA SVOJSTVA POPULACIJA KUKURUZA VISOKOG KVALITETA PROTEINA IZ BANKE GENA

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Izvod

Nutritivna vrednost kukuruza je niska zbog nedostatka nekoliko esencijalnih amino kiselina. Cilj ovog rada je bio da se analiziraju agronomske osobine, sadržaj proteina i amino kiselina kao i fizička svojstva zrna 16 uzoraka iz banke gena koji čine mini *core* za kvalitet, da bi se identifikovale populacije za poboljšanje kvaliteta proteina kukuruza. Hibrid ZP 341 (standard) je bio superioran za većinu testiranih agronomskih svojstava, sa prinosom zrna većim za 24% do 600%. Deset uzoraka kod kojih je sadržaj proteina bio veći od 14% je analizirano na sadržaj aminokiselina i karakteristike zrna. Takođe su utvrđeni genetički odnosi između uzoraka pomoću 30 SSR markera. Svi uzorci su pokazali povećan sadržaj većine esencijalnih amino kiselina. Populacija L492 je imala najveći sadržaj leucina (1.87g $100g^{-1}$suve mase) i fenilalanina (0.68g $100g^{-1}$suve mase), ali i veće sadržaje ostalih aminokiselina ($p<0.05$) u odnosu na ZP341 i analizirane populacije. Klaster analiza zasnovana na SSR markerima je takođe izdvojila populaciju L492 od svih ostalih populacija. U odnosu na ZP 341, populacije iz banke gena su bile inferiorne u masi i dimenzijama zrna ($p<0.05$), ali superiorne u većini parametara tvrdoće zrna ($p<0.05$). Pirsonove korelacije su pokazale nedostatak negativnih korelacija između analiziranih biohemijskih svojstava, što ukazuje na mogućnost poboljšanja kukuruza na više amino kiselina istovremeno. Najbolji način poboljšanja kvaliteta proteina elitnog materijala je putem povratnih ukrštanja, a kako su populacije izabrane prema svojim dobrim opštim kombinacionim sposobnostima (sa IoDent, Lancaster i BSSS), mogle bi da služe za poboljšanje elitnog materijala navedenih heterotičnih grupa.

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