

THE VARIATIONS IN MAIZE GRAIN COMPOSITION INDUCED BY DIFFERENT ENVIRONMENTS

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Nine maize hybrids were grown with the aim to determine variations in chemical composition of the grain. Analyzed hybrids were grown in split-plot experimental design with tree replications in Sremska Mitrovica and Zemun Polje, during the summer of 2010 and 2011. Grain yield, protein, starch, oil, as well as, antioxidants like phytate, soluble phenolics and free sulfhydryl groups (PSH) were analyzed. Unfavorable meteorological conditions affected not only grain yield, but its chemical composition too, by decreasing starch, phenolics and PSH and increasing protein, oil and phytate concentrations. Hybrids from the FAO 600 maturity group achieved higher grain yield during unfavourable conditions, with higher starch and phytate contents and decreased levels of PSH and phenolics. Hybrids from the FAO 500 maturity group were much more stable in respect of protein and oil contents, irrespective to year and location. The grain yield did not have the same source of variations as the factors which determine nutritional quality, an exception being oil content, which decreased with the increase in the grain yield.

KEY WORDS: maize, hybrids, grain composition

INTRODUCTION

Maize grain has a whole range of applications: for feed and food, and as a resource for many industrial and commercial products. Grain composition can be changed in both directions: structure and chemical diversity of starch, protein, oil, secondary metabolites, thus opening new routes for designing novel variations in grain composition (1). On the other hand, chemical composition of grain of hybrids grown on the same location can vary dramatically (2). Mittelman et al. (3) ascertained that there is no association between the nutritional quality and the yield; therefore, the development of cultivars that are superior for high yield and nutritional quality is expected to be feasible.

A typical grain of maize hybrid contains 73% of starch, 9% of protein, 4% of oil and 14% of other constituents (mainly fibers). Two major grain structures, endosperm and germ constitute approximately 80% and 10% of the mature grain dry weight, respectively. The endosperm is mostly starch (approaching 90%), while germ contains high levels

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of oil (30%) and protein (18%) (1). Most of oil in grain is located in embryo (4), so that the increase of oil content in maize is generally connected with the increase in the size of the embryo size. Protein is one of the three most important storage chemical components in maize grain. It is negatively correlated with starch but positively correlated with oil concentration (5, 6). In industrial processing, kernel is degermed and the amylaceous endosperm, which is of the greatest interest to the food industry, is separated from other fractions. Fraction consisting of germ and pericarp is generally used for oil extraction and animal feed due to its high density of nutrients, particularly lipids, proteins, and fibers (4).

Phytic acid is a good chelator of iron ions and thus it can have both antinutritive and antioxidant function (6). More than 80% of grain phytate in maize is located in the germ, with the residue in the aleurone layer, which may contribute to its antioxidative function (7, 8). Various compounds, such as polyphenols and flavonoids have a strong antioxidant function too (9, 10). Antioxidant ability of seeds is highly positively correlated with phenolics content (11, 12). Thioredoxins have a fundamental role in plant tolerance to oxidative stress. They are involved in oxidative damage avoidance by supplying reducing power to reductases detoxifying lipid hydroperoxides or repairing oxidized proteins, as well as in signalling pathways in the plant antioxidant network (13).

Ali et al. (14) and Ngaboyisonga et al. (15) puted out that chemical composition of maize grain could be changed under the negative impact of stress (drought, poor N availability, etc.). Starting from this, an experiment was set up to determine variations in chemical composition (crude protein, starch, oil, as well as, antioxidants like phytate, soluble phenolics and free sulfhydryl groups - PSH) in grain of nine commercial maize hybrids under the influence of different environments.

EXPERIMENTAL

The trial was set up according to split-plot experimental design with three replications at two locations, in Sremska Mitrovica (44° 58' N, 19° 36' E) and Zemun Polje (44° 52' N 20° 20' E), on a slightly calcareous chernozem soil type, during the summer of 2010 and 2011. Nine commercial hybrids as representatives of different maturity groups (FAO 300-600) were used: ZP 341, ZP 427, ZP 434, ZP 555, ZP 560, ZP 600, ZP 606, ZP 666, and ZP 684.

Grain yield, protein, starch, oil, as well as antioxidants like phytate, soluble phenolics and PSH were analyzed in maize grain. Proteins, starch and oil content were determined on a NIR Infratec 1241 Grain analyzer (Foss tecator, Sweden) and presented as percent of dry matter.

For the determination of phytate, soluble phenolics and PSH, maize flour was used (particle size < 500 µm, milled on a Perten 120, Sweden). Four samples of 0.25 g each were treated with 10 ml of bidistilled water for 1 h at room temperature in a rotary shaker. The extract was centrifuged at 14,000 rpm for 15 min (Dynamica – Model Velocity 18R Versatile Centrifuge, Rotor TA15-24-2) and the supernatant was decanted and diluted. Phytate was determined colorimetrically by the method of Dragičević et al. (16) on a Shimadzu UV-1601 spectrophotometer. The method is based on the pink color of the Wade reagent, which is formed upon the reaction of ferric ion and sulfosalicylic acid, and

has an absorbance maximum at $\lambda=500$ nm. The most abundant phenolic compounds in cereals belong to the chemical class of hydroxycinnamic acids, so we choose to determine water soluble phenolics from the same extract as the other antioxidants. They were determined by the method of Simić et al. (17), based on a slightly modified Prussian blue method, where 0.05M FeCl₃ in 0.1M HCl and 0.008 M K₃Fe(CN)₆ were added to the sample solution and the absorbance was measured at 722 nm after 25 min. Phenolic content was expressed in μg of ferulic acid equivalent (FAE). The PSH were determined by method of de Kok et al. (18) by adding 0.2 M potassium phosphate buffer (pH = 8.0) and 10 mM DTNB (5,5'-dithio(2-nitrobenzoic acid)) to the extract and measuring the absorbance at 415 nm.

Statistical analysis of the experimental data included standard deviation (SD), correlation and Principal Component Analysis (PCA) in Minitab 14 (Minitab, 2004).

The meteorological conditions in 2010 and 2011 were quite different (Table 1). The year 2011 was characterised with higher average temperature by about 0.75°C for both locations, as well as by a lower precipitation sum of about 210 mm for both locations. Slightly higher average temperatures were recorded in Zemun Polje. The month with the highest average temperature was August, while the highest average precipitation was recorded in June.

Table 1. Average monthly air temperatures and monthly precipitation sums from April to September 2010 and 2011 in Zemun Polje and Sremska Mitrovica

Month	Temperature (°C)				Precipitation (mm)			
	Srem. Mitrovica		Zemun Polje		Srem. Mitrovica		Zemun Polje	
	2010	2011	2010	2011	2010	2011	2010	2011
April	12.4	13.3	13.2	13.4	51.3	20.6	44	14.9
May	17.1	16.5	17.5	16.8	142.8	56.4	64.1	89.6
June	20.3	20.6	21	21.5	94.2	68.3	167.3	26.2
July	22.9	22.2	23.2	23.3	87.1	93.2	35.6	44
August	22.1	22.8	23.1	23.9	59.4	6.1	68.2	66
September	16.5	20.6	17.6	21.6	74.7	18.8	68	32.6
Average/Sum	18.6	19.3	19.3	20.1	509.5	263.4	447.2	273.3

RESULTS AND DISCUSSION

Grain yield varied between 9.39 t ha⁻¹ (ZP 427, 2010, Sremska Mitrovica) and 13.23 t ha⁻¹ (ZP 684, 2010, Zemun Polje), depending on hybrid (Figure 1). Generally, lower yields were achieved in 2011, with exception of ZP 427, ZP 560 and ZP 600 with the yields which were by about 0.32-1.69 t ha⁻¹ higher in 2011 for both locations. Additionally, higher variations among the hybrids were recorded in 2011, which could be attributed to lower precipitation and higher temperature in that year. This is supported by a clearly negative response of the global yields to increased temperatures (19). The highest average yields achieved ZP 555 and ZP 560. The observed significant and positive correlation between the grain yield, starch oil, phytate and PSH content, as well as a signifi-

cant and negative correlation between the grain yield and protein content (Table 2), indicate the importance of the germ and its components in grain yielding (20, 21).

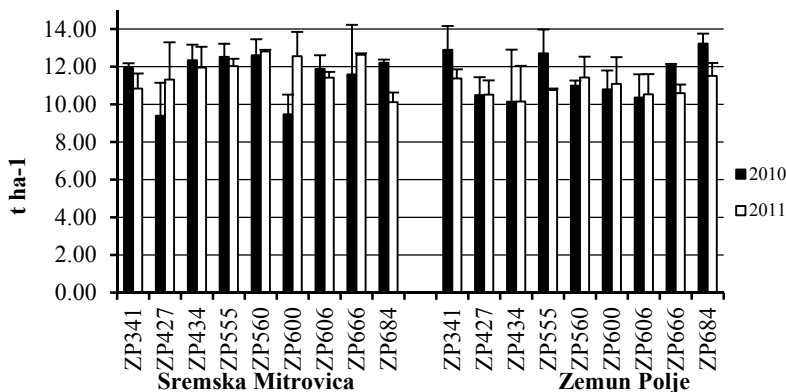


Figure 1. Grain yield of 9 maize hybrids at Sremska Mitrovica and Zemun Polje in 2010 and 2011

It is well known that starch content is important for grain yield and its quality, since the starchy endosperm accounts for 75% of total grain weight (1, 22). Starch content ranged from 69.2% (ZP 606, 2010, Sremska Mitrovica; Figure 2) to 72.1% (ZP 606, 2011, Zemun Polje). The higher variations among the hybrids was recorded in 2011 as less favorable year, and in Zemun Polje, as the location with higher temperatures. It was interesting that the hybrids from FAO 600 maturity group had higher starch content in grain in 2011 at both locations, indicating smaller germ and/or aleurone share in grain. A significant negative correlation between the starch and protein and phenolics (Table 2) was confirmed (5, 6, 23), as well as a significant negative correlation between the starch and PSH.

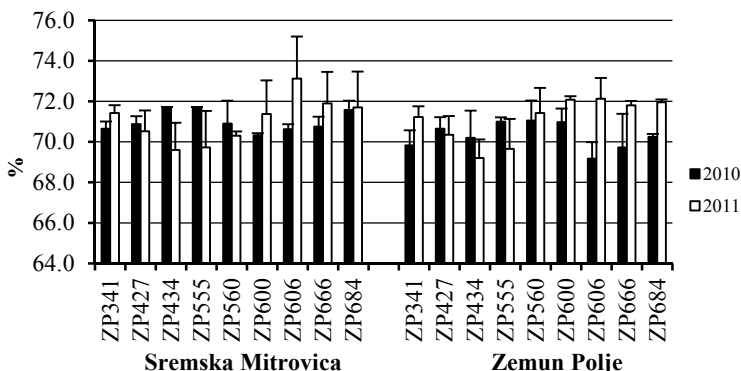


Figure 2. Starch content in grain of 9 maize hybrids at Sremska Mitrovica and Zemun Polje in 2010 and 2011

Protein is a very important storage component in maize grains, too. In general, protein content is negatively correlated with starch and positively with oil content (5, 6, 22, 23).

The protein content ranged from 8.6% (ZP 427, in 2010, Sremska Mitrovica; Figure 3) to 11.8% (ZP 434, 2011, Zemun Polje). A higher protein content in grain was generally achieved in 2011, with the exception of the FAO 600 hybrids, where higher protein content was recorded in Zemun Polje in 2010. This could support the statement that protein content in maize grain is under the high influence of environmental variations (3, 14). Higher variations of the protein content between the genotypes were also recorded in 2011, pointing ZP 555 and ZP 560 as hybrids with the highest protein content in grain and the lowest variability induced by different environments.

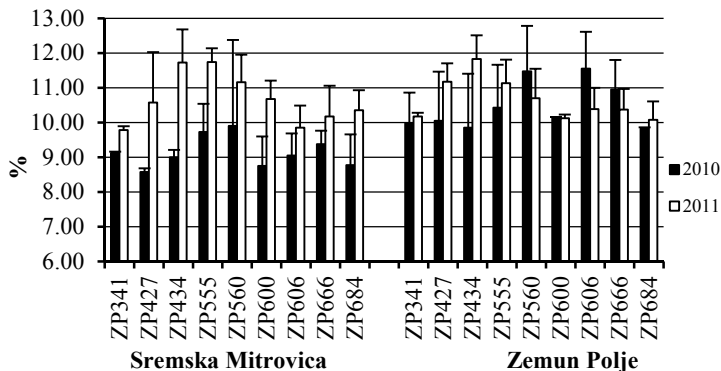


Figure 3. Content of crude protein in grain of 9 maize hybrids at Sremska Mitrovica and Zemun Polje in 2010 and 2011

The oil in maize grain is mainly situated in the germ (4). Its content ranged from 3.3% (ZP 555, 2010, Sremska Mitrovica; Figure 4) up to 4.6% (ZP 600, 2011, Zemun Polje). Similarly to proteins, the oil content had higher values and variation between hybrids in 2011 (except for ZP 606), as well as in Sremska Mitrovica for the hybrids from the FAO group's 400-500, indicating a negative impact of dry season (14). There was no difference in the oil content between the locations for the hybrids from the later maturity groups. The ZP 560 also had the highest average oil content, among all examined hybrids.

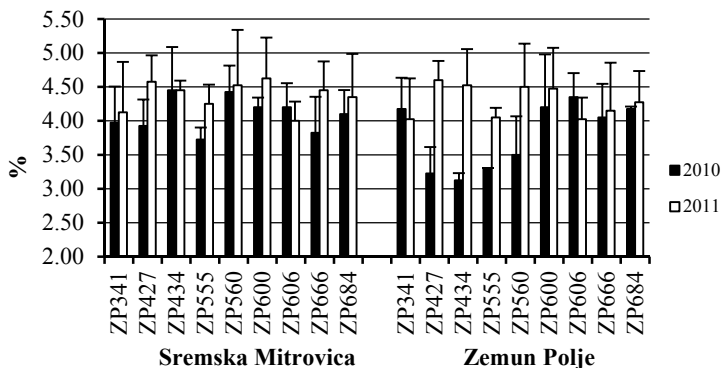


Figure 4. Oil content in grain of 9 maize hybrids at Sremska Mitrovica and Zemun Polje 2010 and 2011

Phytate is one of the most stable components in grain (2, 8). Recently, great importance is given to biological functions of phytic acid and identification of genetic resources and strategies for engineering high-yielding, stress-tolerant, low-phytate germplasm (8, 24). Phytate content in grain varied from 2.47 mg g⁻¹ (ZP 427, 2011, Zemun Polje; Figure 5) to 3.25 mg g⁻¹ (ZP 555, 2011, Sremska Mitrovica). A slightly higher phytate content for all hybrids was obtained for 2011 (similar to the oil and protein contents) in Sremska Mitrovica, except for ZP 666 and ZP 684, which had higher phytate content on the Zemun Polje location. A significant and positive correlation between grain yield and phytate content (Table 2) indicates that the hybrids from the latter maturity groups and with higher yielding potential could have a higher phytate content. Additionally, the negative correlation between the phytate and phenolics could indicate presence of different antioxidative pathways in maize seeds (2, 12). This could be desirable from the aspect of increased nutritive quality by lowering of phytate as anti-nutritive and increasing of phenolics as antioxidants.

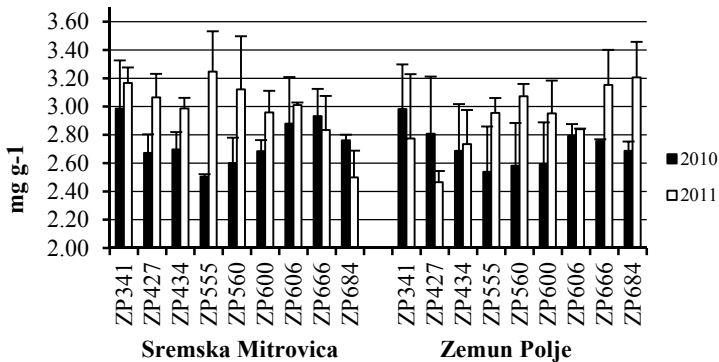


Figure 5. Content of phytate in grain of 9 maize hybrids at Sremska Mitrovica and Zemun Polje 2010 and 2011

Various soluble phenolics have a strong antioxidant function too. The most abundant phenolic compounds in maize belong to hydroxycinnamic acids (9, 10). Rohlig et al. (25) ascertained that growing season is the most prominent impact factor driving variation in phenolics content. Phenolics content in grain of examined hybrids varied from 62.9 µg FAE g⁻¹ (ZP 600, 2011, Zemun Polje) to 488.8 µg FAE g⁻¹ (ZP 600, 2010, Zemun Polje). It is evident that higher variations between the hybrids were recorded in 2011 and at Zemun Polje location, indicating greater extent of stress, which could be confirmed by the meteorological conditions (Table 1). Opposite to the results of Stevanović et al. (23), who done experiments at different ecological conditions, we noticed a high negative correlation between the content of phenolics and starch and phytate in the examined genotypes (Table 2).

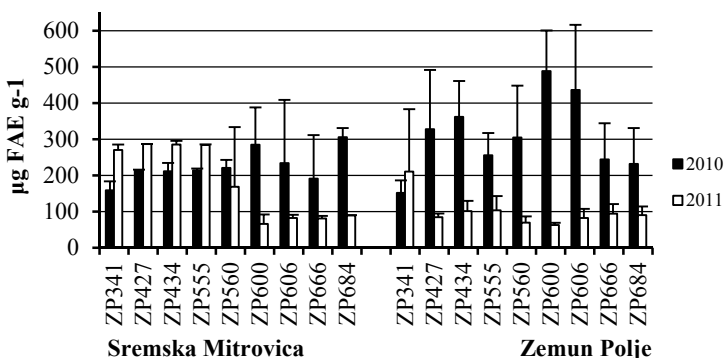


Figure 6. Content of soluble phenolics in grain of 9 maize hybrids at Sremska Mitrovica and Zemun Polje in 2010 and 2011

The thiol redox status is critical in the determination of protein structure, regulation of enzyme activity, control of transcription factor activity (13), as well as in the plant antioxidant network (12). In our research, PSH content ranged from 1587.6 nmol g⁻¹ (ZP 341, 2011, Zemun Polje) to 2853.2 nmol g⁻¹ (ZP 666, 2010, Sremska Mitrovica). Higher PSH values were recorded in 2010 in Sremska Mitrovica, while the variability between the genotypes was present in 2011, indicating high influence of growing season and genotype on variations in PSH content. A high positive correlation between PSH, grain yield and starch content (Table 2) could be tied to its positive role in the yielding potential.

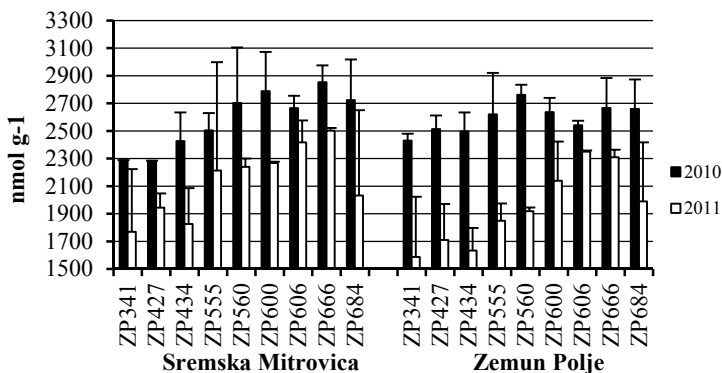


Figure 7. PSH content in grain of 9 maize hybrids at Sremska Mitrovica and Zemun Polje in 2010 and 2011

The projection of variables in PCA indicated that starch and soluble phenolics contributed mostly to PC1 (0.520 and 0.508, respectively; Table 3), while the grain yield and oil contributed mainly to PC2 (-0.560 and 0.527, respectively). This means that the variations of grain yield had no the same source as factors which significantly correlated with

it (Table 2), supporting findings of Mittelman et al. (3) who did not find correlation between the yield and nutritional quality. Moreover, the grain yield was inversely correlated to the oil content, i.e. the increase in the grain yield meant a decrease in the oil content. Total variability explained by the first component was 40.10% and by the second 27.40%, which means that those two dimensions together accounted for around 67.50% of total variability of the observed set of variables.

Table 3. Results of PCA for mineral composition of examined maize hybrids (synthetic variables: PC1 - principal component axis 1 and PC2 - principal component axis 2)

Parameter	PC1	PC2
Grain yield	0.260	-0.560
Starch	0.520	0.273
Proteins	-0.237	-0.404
Oil	0.240	0.527
Phytate	0.330	-0.317
Soluble phenolics	-0.508	0.263
PSH	0.425	0.044
Explained variance	2.810	1.910
Proportion of total variance (%)	40.10	27.40

CONCLUSION

Based on the obtained results it can be concluded that higher temperatures and lower precipitation affected grain yield of examined hybrids, together with the grain chemical composition, by lowering contents of starch, phenolics and PSH and increasing protein, oil and phytate contents. The hybrids from the FAO 600 maturity group achieved higher grain yield during unfavorable conditions, with higher starch and phytate content and decreased PSH and phenolics levels. On the other hand, the hybrids from the FAO 500 maturity group were much more stable for protein and oil content, irrespective to year and location. The grain yield did not have the same source of variations as factors which determine nutritional quality, an exception being the oil content, which decreased with the increase in the grain yield.

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ВАРИЈАЦИЈЕ У САСТАВУ ЗРНА ХИБРИДА КУКУРУЗА УЗРОКОВАНИХ ГАЈЕЊЕМ У РАЗЛИЧИТИМ СРЕДИНАМА

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Девет хибрида кукуруза гајено је са циљем утврђивања варирања у хемијском саставу зрна. Анализирани хибриди су гајени према сплит-плот експерименталном дизајну у три понављања у Сремској Митровици и Земун Пољу, током 2010. и 2011. године. Анализирани су принос зрна, садржај протеина, скроба, уља и антиоксиданата, као што су фитат, растворљиви феноли и слободне сулфхидрилне групе (ПСХ). Неповољни метеоролошки услови су утицали на принос као и на промену хемијског састава зрна, смањујући садржај скроба, фенола и ПСХ и повећавајући концентрацију протеина, уља и фитата. Хибриди из ФАО групе зрења 600 су постигли веће приносе у неповољним условима, са повећаним нивоом скроба и фитата, као и смањеним нивоом ПСХ и фенола. Садржај протеина и уља је био знатно стабилнији код хибрида из ФАО групе 500, без обзира на годину и локацију. Принос зрна није имао исти извор варирања, као и фактори који детерминишу нутритивни квалитет, изузимајући уља чији се садржај смањивао повећањем приноса.

Кључне речи: кукуруз, хибриди, састав зрна

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