# VARIABILITY IN ANTIOXIDANTS IN YELLOW, WHITE, AND RED COLOURED MAIZE GRAIN IN RESPONSE TO DIFFERENT FERTILIZERS

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Maize (Zea mays L.) grain is an important source of nutrients in human diet. The differences in content and relations between certain components of maize grain impact grain colour and its nutritional quality. The objective of the Study was to examine effects of different fertilization systems: mineral fertilizer (urea), organic fertilizer, and biofertilizer on white, yellow, and red coloured maize hybrids, regarding grain yield and variations in content of antioxidants: phytate, phenolic compounds, glutathione, carotenoids (yellow pigment), and reduction capacity of DPPH radical. Two-fold higher average grain yield and double fold lower concentration of phenols and carotenoids were present in 2018, in comparison to drier 2017. The lowest phytate content and the highest values of phenols and DPPH reduction capacity were present in red maize kernel, as a hybrid with the highest yield, while in yellow maize kernel, the highest values of yellow pigment and glutathione occurred. The bio-fertilizer expressed the positive impact on reduction of phytate concentration and increase of phenols concentration in maize grain, while urea increased concentration of yellow pigment and glutathione. Correlation analysis showed that reduction in phytate and carotenoids was significant and positive related with grain yield increase, while phenols showed positive correlation with reduction capacity of DPPH radical. Thus, it was shown that changes in fertilization methods could affect antioxidants status in maize grain, particularly in red coloured maize, which besides high yield potential, possess remarkable higher antioxidant capacity in regard to yellow and white coloured maize.

Key words: Antioxidant capacity, Fertilizing, Grain colour, Grain yield

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#### INTRODUCTION

Maize grain is an important source of nutrients in human diet. Various coloration of maize kernel (white, yellow, orange, red, blue, purple, black, etc.) is related to the presence of pigments, mainly being secondary metabolites, which could have high antioxidant capacity, thus giving additional quality when incorporate into human diet. It is well known that antioxidants express various non-carcinogenic and other anti-inflammatory health benefits. It was proved that maize flour is superior in comparison to wheat flour, due to the higher level of phenolic compounds and antioxidative activity (NIKOLIĆ *et al.*, 2019). Furthermore in experiments with differently coloured maize grain, ŽILIĆ *et al.* (2012) confirmed that phenolic compounds are significantly correlated with antioxidant capacity. Pigment content in maize kernel directly correlates to its antioxidant activity (RODRÍGUEZ et al., 2013).

The presence of high genetic variability among genotypes with various kernel coloration reflects not only on kernel nutritional quality, but also on yield potential. Thus, MAHAN *et al.* (2013) found that hybrids with high antioxidants content in kernels have also low yield, compared to genotypes with low phenolics content. Maize type is an important factor for expression of kernel antioxidant capacity. HU and XU (2011) revealed that antioxidant capacity increases by kernel ripening for yellow and black waxy maize, while for white waxy maize, the antioxidant capacity decreases during maturation.

Growing conditions can contribute to accumulation of various antioxidants and kernel antioxidant activity. BRANKOVIĆ *et al.* (2015) underlined that genotype × environment interaction is an important for accumulation of phytic acid and phenolic substances in wheat kernel, while variation in environmental factors, such as average maximum temperature, sunshine hours sum, average mean temperature and average relative humidity during flowering is important for accumulation of thiolic compounds. In regard to genotype and seasonal influences, it was found that fertilization is not significant for yield of differently coloured maize (OLJAČA *et al.*, 2016). The season plays an important role in variability in grain yield and polyphenol content in grain of wheat grown under low input management (DINNELI *et al.*, 2013).

Fertilization is an important method, for the achievement of high and stable yields, as well as enhanced grain quality of various maize hybrids. Some fertilizers are part of low-input strategy in crop production, supporting efficacy of different genotypes in nutrient absorption. Bio-fertilizers play an important role in yield increase and crops quality, particularly when low-input systems are considered. ROYCHOWDHURY *et al.* (2017) and MISHRA *et al.* (2013) considered bio-fertilizers as one of the best modern tools, which serves as an alternative to mineral fertilizers without harmful impact on the environment, promoting the satisfactory supply of nutrients (phosphorus, calcium, copper, zinc, etc.) to the crops, thus enabling better conditions for their optimal development and growth. ROYCHOWDHURY *et al.* (2017) and WIQAR *et al.* (2013) emphasized that bio-fertilizer, applied sole or in combination with mineral fertilizer is able to significantly increase maize grain yield and other yield parameters, remaining superior over organic fertilizer. Therefore, the aim of the experiment was to

examine the status of antioxidants: phytate, phenolic compounds, glutathione, carotenoids, and reduction capacity of DPPH radical in a kernel of yellow, white and red maize hybrid, affected by the different fertilizer types: mineral (urea), organic, and bio-fertilizer.

#### MATERIALS AND METHODS

The experiment was conducted during the 2017 and 2018 at vicinity of Zemun Polje (44°52'N 20°20'E), on a slightly calcareous chernozem (53.0 % sand, 30.0 % silt, 17.0 % clay, 2.3 % organic matter, 7.0 pH in KCl and 7.17 pH in H<sub>2</sub>O). Maize with different kernel colour: red (ZP 5048c), white (ZP 522b) and standard yellow (ZP 737) was sown in the last decade of April each year. Sowing was performed by randomized complete block design (RCBD), with four replications and elementary plot of 11 m<sup>2</sup> (four lines with 5 m length and 0.7 m inter-row distance). Preceding crop in in both experimental seasons was winter wheat. Prior to sowing, fertilizers were incorporated into soil: urea – Ur (46% N, 200 kg ha<sup>-1</sup>; Elixir Zorka, Šabac, Serbia); bio-fertilizer – BF (3 kg ha<sup>-1</sup>; Team Micorriza Plus, Subotica, Serbia; containing Glomus intraradices and Glomus mosseae and plant growth-promoting rhizobacteria); and organic fertilizer Fertor – OF (2.5 t ha<sup>-1</sup>; Agro Ferticop, Subotica, Serbia; based on chicken manure, containing NPK – 4,5 : 2,7 : 2,3 + 1,1% Mg + 9,3% Ca). One plot was treatment free: control – C, without fertilization. During primary tillage fertilizers were not incorporated in soil.

After harvesting, maize grain yield was measured and calculated with 14% of moisture. The 0.5 kg of grain samples was milled on Perten 120 – Sweden (particle size < 500 μm) and then the content of antioxidants was determined. After the extraction with 5% trichloroacetic acid, and centrifugation, phytic phosphorus (Phy) and total glutathione (GSH) were determined using spectrophotometer (Biochrom Libra S22 UV/Vis Spectrophotometer – Biochrom, UK). Phy was determined by the method of DRAGIČEVIĆ *et al.* (2011). GSH was determined by the method of SARI GORLA *et al.* (1993). Water soluble phenols were determined after the extraction with double distilled water, by the method of SIMIĆ *et al.* (2004). Yellow pigment (YP) was determined by method of VANČETOVIĆ *et al.* (2014). Scavenging activity was determined by the method suggested by AB *et al.* (1998).

#### Statistical analyses

Significant differences between treatment means were determined by the Fisher's least significant difference (LSD) test at the 0.05 probability level, after the analysis of variance (ANOVA) using a two-factorial RCB design. The interdependences between the maize grain yield and examined antioxidants were processed by correlation using Pearson's correlation coefficient, while Principal component analysis (PCA) was used for evaluation of interdependence between applied fertilization treatments and analysed antioxidants (SPSS 15.0 (IBM Corporation, Armonk, New York, USA) for Windows)).

Comparing experimental seasons, 2018 was characterised with higher average temperature and greater precipitation amount, having precipitation peak in June (Table 1). 2017 was prone to drought with higher average temperature and relative low precipitation amount during July-August, i.e. kernel filling period.

	2017 ana 201	o ai Zemai	i i oije						
	Month	IV	V	VI	VII	VIII	IX	X	Aver./ $\Sigma$
T aver.	2017	12.4	18.6	24.4	25.5	25.8	18.4	13.3	19.8
i aver.	2018	18.0	21.7	22.7	23.6	25.7	19.8	15.9	21.1
<u> </u>	2017	47.1	49.2	39.0	26.7	23.7	36.6	62.0	284.3
$\Sigma$ precip.	2018	24.6	39 O	150.1	61.9	44 0	169	20.8	357 3

Table 1. Average monthly air temperatures and precipitation sums for the vegetative period (April–October) of 2017 and 2018 at Zemun Police

### RESULTS AND DISCUSSION

The significant variation in grain yield under the influence of year and its interaction with fertilization and hybrid was noticed (Table 2). Hybrid and its interaction with year and fertilization were responsible for significant variability in phenols content. A year induced significant variability in phytate content in maize kernel in the highest degree. Similarly, PIERGIOVANNI *et al.* (2017) indicated variability in phytate content in bean (*Phaseolus vulgaris* L.) kernels grown in different years and locations. Fertilizer and its interaction with hybrid and year were responsible for the significant variability in GSH content. YP varied significantly, only under the influence of interaction of examined factors. It was proved that not just genotype, but year also play an important role in variability in the antioxidants content in grain of common and durum wheat, such as phytic acid and phenolic compounds (> 92%), although variability of YP was mainly dependable on genotype (BRANKOVIĆ *et al.*, 2015). DRAGIČEVIĆ *et al.* (2017) reported that interaction between production year/cropping management is very important for variability in content of different antioxidants in soybean grain, particularly in stressful environments. DPPH reduction capacity was at least prone to variation, with significant H×F, H×Y and H×F×Y interaction.

Red maize kernel achieved the highest average grain yield (Table 2), as well treatment with organic fertilizer, followed by the bio-fertilizer. ROYCHOWDHURY *et al.* (2017), WIQAR *et al.* (2013), and WU *et al.* (2005) reported higher maize yield with the application of organic and/or bio-fertilizer, due to the improved uptake of macro-nutrients. In our experiment, Ur expressed the highest influence on GSH and YP content, while BF treatment had the highest impact on phenols accumulation in maize grain.

Yellow kernel had the highest content of phytate, GSH, and YP, while red kernel had higher values of phenols and DPPH reduction capacity. The highest phytate content and value of DPPH reduction capacity was in the control treatment. Irrespective to the minor variations in average Phy content, according to the data present in Figure 1, BF tends to reduce accumulation of Phy mainly in white and yellow maize kernel (with values of 2.56 and 2.62 mg g<sup>-1</sup>, respectively). Similarly to this, the OF expressed the same influence to some extent in red kernel (with value 2.61 mg g<sup>-1</sup>), what could be considered as a positive impact, due to the phytate ability to bind minerals (DWIVEDI *et al.*, 2012). As expected, YP had the lowest average values in white kernel (1.14 µg g<sup>-1</sup>) and the highest average values in yellow kernel (12.45 µg g<sup>-1</sup>). The highest influence on YP increase expressed Ur, mainly in yellow kernel, while in red kernel the highest YP content was in the control (13.41 and 10.52 µg g<sup>-1</sup>, respectively). BF mainly induced increase of phenols in white and yellow kernel (127.42 and 57.71 µg g<sup>-1</sup>, compared to control), but in red

kernel the highest value was noticed in the control (539.30  $\mu g$  g<sup>-1</sup>). HU and XU (2011) also determined high variability in a content of total phenols among kernels of differently coloured maize.

Table 2. Analysis of variance for the effect of hybrid, fertilization and year on grain yield (GY) and contents of phytic P (Phy), phenols, glutathione (GSH), yellow pigment (YP) and DPPH reduction capacity (DPPH) in maize with different grain colour

Sou			GY (t	ha <sup>-1</sup> )	Phy (n	ng g <sup>-1</sup> )	GSH (nı	mol g <sup>-1</sup> )	Phenols	(μg g <sup>-1</sup> )	ΥP (μ	ıg g <sup>-1</sup> )	DPPF	H (%)
ot varia		df <sup>(b)</sup>	$MS^{(c)} \\$	LSD <sub>0.05</sub>	MS	LSD <sub>0.05</sub>	MS	LSD <sub>0.05</sub>	MS	$LSD_{0.05}$	MS	$LSD_{0.05}$	MS	$LSD_{0.05}$
H <sup>(</sup>	e)	2	6.01	2.55	77865*(a)	288.10	610379	812.20	444567*	269.60	65362	288.40	70598	275.40
$\mathbf{F}^{(i)}$	f)	3	0.17	2.59	$0.037^{*}$	0.17	$666172^{*}$	807.90	6742	183.70	2.60	6.46	24.7	6.58
<b>Y</b> (	g)	1	514.07*	1.05	162375*	403.00	231076	818.50	194703	372.50	419	402.00	4	385.10
НХ	F	11	1.57	2.67	$0.038^{*}$	0.16	632577*	812.40	90991*	118.30	105.3*	4.36	126.60*	4.03
Нх	Y	5	113.88*	0.73	$0.1337^{*}$	0.13	707986*	804.00	271619*	59.07	352.40*	1.39	208.20*	4.72
Fx	Y	7	$73.95^{*}$	1.07	$0.027^{*}$	0.12	$601564^{*}$	814.00	34337*	177.80	$64.80^{*}$	5.89	14.4	6.85
H x		23	25.023*	0.72	0.049*	0.097	654782*	807.7	64094*	35.64	78.19*	1.36	83.85*	1.52
CV	J (%	) <sup>(h)</sup>	3.76		2.78		4.82		2.34		1.86		1.16	
	В	F	6.923		2.63		947.6		376.77		7.17		91.88	
	Į	Jr	6.874		2.73		1437.1		358.03		8.17		92.90	
e e	О	rg	7.042		2.72		974.0		329.05		8.06		90.56	
Average	(	2	6.846		2.76		978.3		328.05		8.04		93.91	
¥.	Wł	nite	6.88		2.68		1053.8		297.38		1.14		91.79	
	Yel	low	6.58		2.79		1294.7		207.25		12.45		87.66	
	R	ed	7.44		2.66		904.3		539.30		10.00		97.48	

 $<sup>^{(</sup>a)}$ Significant at the 5% probability level;  $^{(b)}$ df: degrees of freedom;  $^{(c)}$ MS: mean squares;  $^{(d)}$ LSD<sub>0.05</sub>: least significant difference at the 0.05 probability level;  $^{(e)}$ H: hybrid;  $^{(f)}$ F: fertilization;  $^{(g)}$ Y: year;  $^{(h)}$ CV: coefficient of variation

The highest average GSH content was noticed in Ur treatment in white and yellow kernel (1113.70 and 1319.16 nmol g<sup>-1</sup>, respectively; Figure 2), while in red kernel values varied slightly and the highest value was achieved in OF treatment (941.56 nmol g<sup>-1</sup>). DPPH reduction capacity varied similarly to the phenols content in white and yellow kernel among fertilizer treatments, having the highest average values in the BF treatment (with values of 95.65% and 98.99%, respectively). SARUHAN GULER *et al.* (2016) ascertained that maize inoculation with Trichoderma atroviride provided decrease of lipid peroxidation and increased activity of antioxidant enzymes. However, greater variations of DPPH reduction capacity were present in yellow kernel, with the highest average value of 96.1% obtained in the control, while lower variability and higher average value (91.8%) was noted in white kernel supporting statement of

NIKOLIĆ *et al.* (2019) that maize flour, particularly from white kernels is the great source of phenolic compounds, with high antioxidant activity.

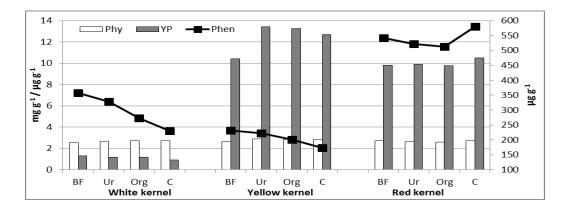


Figure 1. The effect of different fertilizers (BF – bio-fertilizer, Ur – urea, Org – organic fertilizer, C – control) on variation in phytic P (Phy), yellow pigment (YP) and soluble phenols (Phen) in maize with white, yellow and red kernel (average for 2017–2018).

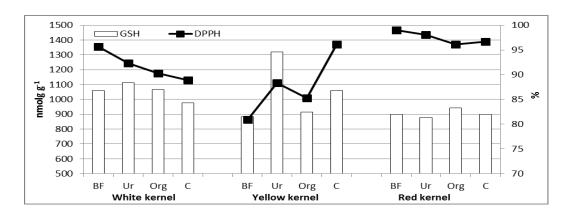


Figure 2. The effect of different fertilizers (BF – bio-fertilizer, Ur – urea, Org – organic fertilizer, C – control) on variation in total glutathione (GSH) and reduction capacity of DPPH radical (DPPH) in maize with white, yellow and red kernel (average for 2017–2018).

-0.047

-0.909\*

	White kernel	Yellow kernel	Red kerne		
		Grain yield			
Antioxidants					
	0 = 10*(-)	0.071*	0.712*		
Phytic P	$0.719^{*(a)}$	-0.951*	-0.543*		
Phenols	-0.591*	-0.266	-0.217		

 $-0.749^*$ 

 $-0.735^*$ 

-0.919\*

-0.559\*

Table 3. Correlation between maize grain yield and examined antioxidants

Yellow pigment

DPPH reduction capacity

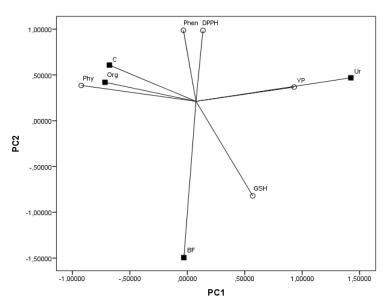


Figure 3. Principal Component Analysis for phytic P (Phy), phenols (Phen), yellow pigment (YP), glutathione (GSH), and reduction capacity of DPPH radical (DPPH) in maize kernel affected by different fertilizers: BF – bio-fertilizer, Ur – urea, Org – organic fertilizer, C – control

Connection between grain yield and examined antioxidants exhibited significant and negative correlation with phenols, YP, and DPPH reduction capacity in white kernel, as well as with phytic P, GSH, YP and DPPH reduction capacity in yellow kernel (Table 3). The negative correlation between grain yield and phytic P and phenols, observed in yellow kernel hybrid could be considered as a positive trait, due to the fact that phytates and phenols mutually obstruct bioavailability of certain minerals from bean kernels (DWIVEDI *et al*, 2012). Positive correlation was

<sup>&</sup>lt;sup>(a)\*</sup>Correlation is significant at 0.05 probability level (Pearson correlation)

present between grain yield and phytate content in white kernel. The positive correlation between grain yield and GSH was present in red kernel, while correlation with phytate and DPPH reduction capacity was negative. MESAROVIĆ *et al.* (2018) specified that correlation between antioxidants content and kernel yield in sweet maize hybrids is highly variable. They emphasized the importance of phenolics for the antioxidant capacity and yield potential.

Principal Component Analysis revealed that PC1 axis participated with 61.0% in total variability and PC2 with 38.3% in total variability. Phenols and DPPH reduction capacity correlated positive with 1<sup>st</sup> axis, similarly to the results of ŽILIĆ *et al.* (2012) who obtained high correlation between phenolics and antioxidant capacity in maize grain with various colour. GSH correlated negative to the same axis. YP correlated positive with 2<sup>nd</sup>, while phytate correlated negative with the same axis. Hence, RODRÍGUEZ et al. (2013) found direct connection between antioxidant capacity of maize kernels and pigment content, i.e. hydrophilic fraction, mainly consisting from phenolics and proteins. Figure 3 shows that phytic P varied mainly in C and OF treatments, while Ur expressed the highest influence on YP variability. BF expressed the highest impact on GSH variability.

### **CONCLUSION**

Based on the results, it can be concluded that grain yield and examined antioxidants exhibited significant variability across variation sources: mainly of genotype, and then of fertilizer type, year, and their interaction. High variability among examined hybrids and antioxidants was detected. Red kernel was superior, high in YP and phenols, although white kernel was high in phytate, phenols and GSH, and yellow kernel was richer in YP and GSH. Therefore, all three hybrids can be considered as a good source of antioxidants. Correlation confirmed the important connection of yield potential and antioxidants. Each of the applied treatments expressed particular impact on antioxidants variability: OF for phytate, Ur for YP, and BF for GSH concentration. Thus, it can be assumed that accumulation of some antioxidants in maize kernel can be increased by application of selected fertilizers, particularly in red coloured maize, which possess remarkable higher antioxidant capacity in regard to yellow and white colour maize.

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# VARIJABILNOST ANTIOKSIDANTA U ZRNU KUKURUZA ŽUTE, BELE I CRVENE BOJE KAO ODGOVOR NA RAZLIČITA ĐUBRIVA

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#### Izvod

Zrno kukuruza (Zea mays L.) je važan izvor hranljivih materija u ljudskoj ishrani. Razlike u sadržaju i odnosima između pojedinih komponenti utiču na boju zrna i njegov nutritivni kvalitet. Cilj eksperimenta je bio da se ispita uticaj različitih sistema đubrenja: mineralnog đubriva (uree), organskog đubriva i bio-đubriva na prinos zrna i varijacije u sadržaju antioksidanata kao što su fitat, fenolna jedinjenja, glutation, karotenoidi (žuti pigment) i redukcioni kapacitet DPPH radikala u belom, žutom i crvenom hibridu kukuruza. Dvostruko veći prosečni prinos zrna i duplo manja koncentracija fenola i žutog pigmenta bili su prisutni u 2018. godini u odnosu na sušnu 2017. Najmanji sadržaj fitata i najveće vrednosti fenola i redukcionog kapaciteta DPPH bili su detktovani u zrnu crvenog kukuruza, kao hibrida sa najvećim ostvarenim prinosom, dok su u zrnu žutog kukuruza zabeležene najveće vrednosti karotenoida i glutationa. Bio-đubrivo je pokazalo pozitivan uticaj na smanjenje koncentracije fitata i povećanje koncentracije fenola u zrnu kukuruza, dok je urea povećala koncentraciju žutog pigmenta i glutationa. Korelaciona analiza je pokazala da je smanjenje fitata i karotenoida značajno i pozitivno povezano sa povećanjem prinosa zrna, dok su fenoli visoko i pozitivno korelisali sa redukcijskim kapacitetom DPPH radikala. Tako se pokazalo da promene u uslovima proizvodnje, kao što su različiti načini đubrenja mogu uticati na status antioksidanata u zrnu kukuruza, posebno crveno obojenog, koji pored visokog potencijala prinosa, poseduje značajno veći antioksidativni kapacitet u odnosu na kukuruz žutog i belog zrna.

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