

## EVALUATION OF MORPHOLOGICAL AND KERNEL MICRONUTRIENT TRAITS IN MAIZE LANDRACES

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### Abstract

Maize grains contain high level of carotenoids and tocopherols compared with other cereals. Among carotenoids the  $\beta$ -carotene has the highest activity and is considered important in breeding programs of biofortified crops. Changes in carotenoids content in the maize grain could be influence of genotype x environment interaction, or effect of existing relationship between the color of the endosperm and the presence of carotenoids. This research was performed to estimate differences in morphological traits, grain content of tocopherols ( $\alpha$ -,  $\beta+\gamma$ -,  $\delta$ -),  $\beta$ -carotene, lutein and zeaxantin in local and introduced populations from Maize Research Institute "Zemun Polje" gene bank. Micronutrient content was detected by using high-performance liquid chromatography (HPLC). Coefficient of variation for morphological traits was less than 10%, except for grain yield per plant which vary about 18,6%.  $\alpha$ -tocopherol content was in the range from 1.04-8.42  $\mu\text{gg}^{-1}$  DW, and  $\beta$ -carotene content varied from 0.26 to 7.95  $\mu\text{gg}^{-1}$  DW.  $\delta$ -tocopherol was in significant correlation with number of kernels per row ( $r=0.700^{***}$ ), and  $\beta+\gamma$ -tocopherol was in significant negative correlation with plant and ear height ( $r=-0.601^{***}$ ;  $r=-0.591^{**}$ ). Correlations between morphological traits and  $\alpha$ -tocopherol were weak and without significance. Kernel color was significantly correlated with the content of zeaxantin ( $r=0,590^{***}$ ) and  $\beta$ -carotene ( $r=0,398^*$ ). For biofortification purposes a pool of 11 landraces with increased content of both,  $\beta$ -carotene and  $\alpha$ -tocopherol, will be created based on obtained results.

**Key words:**  $\beta$ -carotene, grain, tocopherol, populations, *Zea mays* L.

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## Introduction

Maize grain contains considerable amounts of carotenoids (Wurtzel, 2004) and has a greater total phenolic content and total antioxidant activity than wheat, oats or rice (Adom and Liu, 2002). Kernel could be of different color, such as white, yellow, orange, purple, black and mixed varieties. In addition to its attractive colors, pigmented maize grain is rich in phytochemicals like phenolic compounds, carotenoids and flavonoids.

Carotenoids and tocopherols typically found in maize include  $\alpha$ -carotene,  $\beta$ -carotene, cryptoxanthin, lutein, zeaxanthin, and  $\alpha$ -,  $\beta$ -, and  $\gamma$ -tocopherols. About 50 carotenoids have pro-vitamin A activity, and the  $\beta$ -carotene has the highest activity and for this reason, has been considered of high interest in breeding of biofortified crops. According to Harvest Plus project, 15  $\mu\text{g/g}$  of provitamin A carotenoids content is the target value in maize, and half of that is acceptable for the beginning of breeding program (Pixley et al., 2013). For breeding purposes and biofortification improvement in maize, it is necessary to have naturally occurred variability, and the first step is to identify concentration of micronutrient of interest, e.g. carotene, xanthophylls, tocopherol etc.

Maize (*Zea mays* L.) grain is important sources of vitamin E for food and feed (Rocheford et al., 2002). They exist as a family of four derivatives each ( $\alpha$ -,  $\beta$ -,  $\gamma$  and  $\delta$ ), which differ in the number and position of methyl substitutions in the chromanol ring. Tocopherols are well recognized as antioxidants in vegetable oils, and their presence increases the stability of lipid membranes, protecting against autoxidation and preventing damage to tissues (Sattler et al., 2004; Traber et al., 2007).

Landraces are dynamic populations of plant with a historical origin, identity, genetically diverse, result of both natural and

human selection (Bellon and van Etten, 2013). In general, cereal landraces and old varieties are among the best sources of phytonutrients accompanied with optimal micronutrient concentrations. Nevertheless, while traits associated with yield and resistance has been the focus of most research, quality traits that are dependent on chemical composition are less studied.

The aim of the present study was to investigate micronutrient content in dark yellow and orange colored kernels of local and introduced maize landraces, in order to create a pool which will be used in biofortification breeding programs.

## Material and methods

In this study 29 accessions with orange kernel (Drinić Mladenović et al., 2016) from Maize Research Institute Zemun Polje (MRIZP) genebank were analyzed for morphological traits, their carotenoid and tocopherol content and composition. The accessions were multiplied in the experimental field of MRIZP and planted in two rows, with spacing of 0.7m between rows and 0.4m between hills. Plots were overplanted and thinned to two plants per hill after seedling establishment. Five plants from each row were selfed and the rest ears were allowed to open-pollinate. Following morphological traits were measured on ten randomly chosen plants per plot and averaged: plant height (PH-cm), ear height (EH-cm), leaf width (LW-cm), leaf length (LL-cm), number of leaves above the uppermost ear including ear leaf (NoL). Ear leaf length was measured in cm after pollination; also, the width of the same leaf was measured at the widest point, in cm (LW, Tollenar et al., 2004). Ear length (EL-cm), number of rows per ear (NRE) and number of kernels per row (NKR) were measured on 10 open pollinated ears after harvesting. Kernel

types (Ktyp) and kernel colors (Kcol) were evaluated according to IBPGR descriptors.

Maize grains from selfed ears were milled into flour (particle size less than 500µm), and used for the analyses of carotenoid and tocopherol content. Standards for carotenoids (lutein, zeaxantin and β-carotene) and tocopherol (α-, γ+β and γ) were produced by Sigma Aldrich (Germany).

Carotenoid content and composition was determined using high performance liquid chromatography (HPLC), slightly modified method proposed by Rivera and Canela, (2012). Briefly, 0.15g of maize flour was extracted twice with 5mL of the methanol-ethyl acetate (6:4, v/v) for 30 min at room temperature. After centrifugation (3000 rpm, for 5 min), the extracts were evaporated under the stream of nitrogen to the dryness and dissolved in 1mL of the mobile phase, and filtered through syringe filter for analysis by DionexUltiMate 3000 liquid chromatography system (Thermo Scientific, Germany). Chromatograms were generated at 450nm and 470nm and carotenoids were identified and quantified by comparing with appropriate standards, and expressed as

µg/g of dry weight (DW).

Tocopherols (α-, γ+β and γ) were extracted using method described by Gliszczyńska-Świgło et al. (2007). Approximately 0.2 g of sample was mixed with 2-propanol, homogenized for 30 min, and centrifugated at 3000 rpm for 5 min. After filtration, an aliquot of the supernatant was injected to DionexUltiMate 3000 liquid chromatography system. The wave lengths for excitation and emission were at 290 nm and 325 nm. Identification and quantification of the tocopherols content were done by comparison to the corresponding standards, and expressed in µg/g of dry weight.

Analysis of variance according to a complete randomized block design was performed separately for all tested traits, and where main effect was significant (p≤ 0.05), least significant difference (LSD) was used to compare means. All analyses were accomplished using M-STAT-C package (Bricker, 1989). The correlations were determined by Pearson’s correlation analysis. The calculation of correlation coefficients was done in Microsoft Excel.

Table 1. Average, minimum and maximum value of examined traits in maize landraces  
Tabela 1. Prosečne, minimalne i maksimalne vrednosti ispitivanih svojstava kod populacija kukuruza

Trait	Mean	Min	Max	Cv (%)
Plant height (cm)	216.73	128.50	270.75	6.03
Ear height (cm)	98.22	49.75	147.75	9.06
Number of leaves above ear	6.14	4.85	8.50	4.71
Leaf length (cm)	84.46	61.40	103.55	3.52
Leaf width (cm)	9.78	7.32	12.10	6.66
Ear length (cm)	15.54	10.10	20.05	4.33
Number of rows per ear	13.18	10.80	15.50	3.98
Number of kernels per row	30.51	21.83	38.10	3.42
Grain yield per plant (g)	51.98	29.48	112.76	18.60

## Results and discussion

Maize landraces represent natural variability, both, in agronomically important traits, and also in micronutrients, which have decreased during the breeding of modern high yielding hybrids. For the present study, we have chosen local landraces, collected in the former Yugoslavia territory, and landraces from abroad, with predominantly orange color and flint type of the kernel (Mladenović Drnić et al., 2016). Coefficient of variation for morphological traits was between 3.42% (number of kernels per row) and 9.06% (ear height), (Table 1). Correlations between all morphological traits were significant, except interrelations with number of kernels per row (data not shown).

Maize exhibits great natural variation considering carotenoids and tocopherol contents in the kernels. Generally, two classes of carotenoids, carotens and xanthophylls are responsible for the color of maize kernel, and their concentration is influenced by genetic and environmental factors. Provitamin A carotenoids ( $\beta$ -cryptoxanthin,  $\alpha$ - and  $\beta$ -carotene) usually represent 10-20%, whereas zeaxanthin and lutein are dominant carotenoids with 30-50% of total content in maize grain (Ortiz-Monasterio et al., 2007).

In the present study,  $\beta$ -carotene content varied from 0 (white kernel) to  $7.95\mu\text{gg}^{-1}$  DW, with the average content of  $2.87\mu\text{gg}^{-1}$  DW. Out of all samples, 17 landraces had  $\beta$ -carotene content bigger than  $2\mu\text{gg}^{-1}$  DW (Table 2). Significant variability was detected for lutein and zeaxanthin content, e.g. from 0 to 15.49 and  $40.86\mu\text{gg}^{-1}$  DW, respectively. In general, biofortification comprehend increasing in nutrient content in food (Pfleffer and McClafferty, 2007). During last decades, maize is getting more importance because of  $\beta$ -carotene, precursor of vitamin A in cereals

(Welch, 2002). However, larger quantities of xanthophylls (lutein and zeaxanthin) in maize kernel are of less importance (Kuhnen et al., 2009). These carotenoids are with well-known antioxidant activity, but they are non-provitamin A carotenoids. Maize kernels with intense yellow and orange color reflect increased concentration of xanthophylls (Rivera and Canela, 2012). In our study, the strongest correlation for kernel color was obtained with zeaxanthine content ( $r=0.590^{***}$ ), and weaker, but significant with  $\beta$ -carotene content ( $r=0.398^*$ ). However, higher level of zeaxanthine was correlated with increased concentration of  $\beta$ -carotene ( $r=0.566^{**}$ ), (Table 3.). Similar relation was obtained between color and  $\beta$ -carotene, and zeaxanthin content, respectively ( $r=0.36$ ;  $r=0.21$ ), (Harjes et al., 2008).

Considering previous studies maize grain color depends on double bonds and the various functional groups in the carotenoid molecule, and could not be directly connected with carotenoid content. However, chosen accessions were with dark yellow and orange grain color, and it could be expected that they have higher concentrations of  $\beta$ -carotene.

As it was previously shown (Combs and Combs, 1985), the most abundant form of tocopherols in maize kernels were  $\gamma+\beta$ . The content varies from 13.05 to  $37.27\mu\text{gg}^{-1}$  DW (Table 4). Increased  $\alpha$ -tocopherol is commercially desirable because of its higher anti-oxidant activity than  $\gamma$ -tocopherol (Rocheford et al., 2002). Examined landraces showed increased level of  $\alpha$ -tocopherol (from 1.04 to  $8.43\mu\text{gg}^{-1}$  DW), with noticeable higher content in introduced populations. The landraces number 1 and 12 had the highest content of  $\gamma+\beta$ , as well as  $\alpha$ -tocopherol. However, within introduced landraces, the highest  $\alpha$ -tocopherol content in kernel was in

KERNEL MICRONUTRIENT CONTENT IN MAIZE LANDRACES 39-48

Table 2. Kernel content of  $\alpha$ -,  $\beta+\gamma$ -,  $\delta$ -tocopherol (in  $\mu\text{g g}^{-1}\text{DW}$ ) in 29 maize landraces  
 Tabela 2. Sadržaj  $\alpha$ -,  $\beta+\gamma$ -,  $\delta$ -tokoferola u zrnu ( $\mu\text{g g}^{-1}\text{suve mase}$ ) kod 29 populacija kukuruza

Landraces	$\alpha$ -tocopherol	$\beta+\gamma$ -tocopherol	$\delta$ -tocopherol
1	7.90 <sup>ab</sup>	31.33bc	2.21cd
2	3.11k	28.69cde	6.00a
3	4.37fghi	22.02ghijkl	1.91fg
4	3.41ijk	22.98fghij	1.49jk
5	4.63efg	24.29efgh	1.63ij
6	1.57mn	30.57bcd	4.38b
7	1.04n	19.15ijklmn	1.57ijk
8	3.07k	34.40ab	2.00ef
9	3.57hijk	13.26o	0.97pqr
10	4.17fghij	25.74defg	1.72hi
11	6.97bc	30.06bcd	1.47kl
12	7.71ab	37.27a	2.08de
13	6.53cd	15.55mno	1.32lm
14	6.24cd	23.13fghi	1.88fg
15	4.51fgh	19.99hijklm	1.1 nop
16	1.99mn	21.84ghijkl	1.29m
17	3.02kl	17.90klmno	1.00pq
18	3.76ghijk	14.23no	0.93qr
19	4.94ef	23.97efghi	1.79gh
20	4.72efg	17.49lmno	0.83rs
21	4.70efg	15.87mno	1.72hi
22	1.51mn	24.11efghi	2.36c
23	3.16k	22.70ghijk	2.26c
24	5.57de	28.00cdef	2.07de
25	8.09a	15.10mno	1.04opq
26	3.21jk	19.63hijklm	1.25mn
27	8.40a	17.94ijklmno	1.19mno
28	2.04lm	17.73klmno	1.05opq
29	4.77	14.54no	0.75s
LSD	0.98	5.08	0.159

LSD (last significant difference) between means using *t* tests where  $p > 0.05$

Table 3. Association of grain quality traits of 29 maize landraces  
 Tabela 3. Međuzavisnost svojstava kvaliteta zrna kod 29 populacija kukuruza

	Kcol	$\delta$ -toco.	$\gamma$ + $\beta$ toco.	$\alpha$ -toco.	lutein	zeaxantin	$\beta$ -caroten
Ktype	0.563**	0.072	-0.040	-0.228	-0.489**	0.216	0.072
Kcol		-0.085	-0.074	-0.097	-0.288	0.590***	0.398
$\delta$ -toco.			0.559**	-0.212	-0.142	-0.231	-0.381*
$\gamma$ + $\beta$ toco.				0.149	-0.030	-0.319	-0.229
$\alpha$ -toco.					0.267	0.075	0.500**
lutein						-0.082	0.040
zeaxantin							0.566**

\*, \*\* and \*\*\* means significant at  $p < 0,05$ ;  $0,01$  and  $0,001$ , respectively

populations 27 and 25, which have relatively low content of  $\gamma$ + $\beta$  tocopherols in maize grain. The level of most important,  $\alpha$ -tocopherol is about 20% of total tocopherols in maize (Rocheferd, 2002), and its higher levels can be obtained through either utilization of existing natural variation or by genetic engineering (Hallauer and Miranda, 1995). In our study, the only significant correlation was obtain between  $\alpha$ - and  $\delta$ -tocopherol ( $r=0.559^{**}$ ). In the study of maize populations, correlations between  $\alpha$ - and  $\gamma$ -tocopherol was low and negative ( $r=-0.140^{**}$ , Galili et al., 2002). All the other correlations between carotenoids and tocopherols, except between  $\beta$ -caroten and zeaxantin, ( $r=0.566^{**}$ ) were non significant (Table 3), like in the study of maize sweet and dent lines (Kurilich and Juvik, 1999). One of possible conclusion is that content of these micronutriens are under independent regulation and that increased level could be bred simultaneously.

Although the information about nutritional content of maize kernel is scarce, inbreds and populations are increasingly used for biofortification. Obtained results

from present study will contribute to growing interest for exploitation of maize landraces with increased nutritional properties due to society demands.

### Conclusions

The landraces could be chosen to create a pool for breeding for increased content of  $\beta$ -carotene and  $\alpha$ -tocopherol, on the basis of obtained results:

- 17 landraces were separated according to  $\beta$ -carotene content higher than  $2 \mu\text{gg}^{-1}$  DW; out of them, landraces number 10,13,18 and 19 showed no significant difference among each other;
- 16 landraces have  $\alpha$ -tocopherol content higher than  $4 \mu\text{gg}^{-1}$  DW; out of them landraces number 3, 5, 15, 19, 20, 21, 24 and 29 were with similar content.
- For simultaneously increased content of  $\beta$ -carotene and  $\alpha$ -tocopherol, pool for breeding purposes could be created from 11 landraces 3, 5, 10, 12, 13, 15, 19, 20, 24, 25 and 27.

KERNEL MICRONUTRIENT CONTENT IN MAIZE LANDRACES 39-48

Table 4. Kernel content of  $\beta$ -carotene, lutein and zeaxantin (in  $\mu\text{gg}^{-1}$  DW) in 29 maize landraces  
 Tabela 4. Sadržaj  $\beta$ -karotena u zrnu ( $\mu\text{g g}^{-1}$  suve mase) kod 29 populacija kukuruza

Landraces	$\beta$ -carotene	lutein	zeaxantin
1	1,59 <sup>jk</sup>	9,43 <sup>b</sup>	7,05 <sup>jk</sup>
2	1,09 <sup>lm</sup>	1,50 <sup>jk</sup>	8,96 <sup>hij</sup>
3	3,38 <sup>f</sup>	9,09 <sup>b</sup>	10,61 <sup>gh</sup>
4	2,34 <sup>hi</sup>	7,23 <sup>c</sup>	9,475 <sup>hij</sup>
5	3,01 <sup>fg</sup>	9,14 <sup>b</sup>	30,05 <sup>b</sup>
6	0,55 <sup>n</sup>	5,69 <sup>de</sup>	4,14 <sup>lmn</sup>
7	0,68 <sup>mn</sup>	3,80 <sup>fg</sup>	1,51 <sup>no</sup>
8	1,06 <sup>lm</sup>	2,91 <sup>ghi</sup>	1,89 <sup>mno</sup>
9	4,39 <sup>de</sup>	9,99 <sup>b</sup>	19,86 <sup>d</sup>
10	4,77 <sup>cd</sup>	7,61 <sup>c</sup>	15,19 <sup>ef</sup>
11	1,31 <sup>kl</sup>	7,03 <sup>c</sup>	3,131 <sup>mn</sup>
12	4,07 <sup>e</sup>	7,35 <sup>c</sup>	1,87 <sup>mno</sup>
13	4,79 <sup>cd</sup>	15,49 <sup>a</sup>	10,38 <sup>ghi</sup>
14	0,74 <sup>mn</sup>	5,25 <sup>e</sup>	2,281 <sup>mno</sup>
15	2,04 <sup>ij</sup>	14,48 <sup>a</sup>	7,86 <sup>ij</sup>
16	2,61 <sup>gh</sup>	2,78 <sup>ghij</sup>	14,02 <sup>ef</sup>
17	2,25 <sup>hi</sup>	4,36 <sup>ef</sup>	4,26 <sup>lm</sup>
18	4,78 <sup>cd</sup>	1,45 <sup>jk</sup>	40,86 <sup>a</sup>
19	4,83 <sup>cd</sup>	1,60 <sup>ijk</sup>	13,03 <sup>fg</sup>
20	4,49 <sup>de</sup>	4,71 <sup>ef</sup>	7,16 <sup>jk</sup>
21	1,43 <sup>kl</sup>	6,8 <sup>cd</sup>	4,59 <sup>kl</sup>
22	0,00 <sup>o</sup>	0,0 <sup>l</sup>	0,0 <sup>o</sup>
23	0,57 <sup>n</sup>	2,95 <sup>gh</sup>	2,05 <sup>lmno</sup>
24	5,40 <sup>b</sup>	1,93 <sup>hijk</sup>	16,47 <sup>e</sup>
25	7,95 <sup>a</sup>	1,24 <sup>kl</sup>	22,75 <sup>c</sup>
26	5,09 <sup>bc</sup>	1,74 <sup>hijk</sup>	10,40 <sup>ghi</sup>
27	5,52 <sup>b</sup>	2,49 <sup>ghijk</sup>	4,48 <sup>klm</sup>
28	1,49 <sup>kl</sup>	2,19 <sup>hijk</sup>	7,14 <sup>jk</sup>
29	1,14 <sup>klm</sup>	2,19 <sup>hijk</sup>	9,28 <sup>hij</sup>
LSD	0,48	1,337	2,692

LSD (last significant difference) between means using *t* tests where  $p= 0.05$

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## EVALUACIJA MORFOLOŠKIH OSOBINA I MIKRONUTRIENATA ZRNA KOD POPULACIJA KUKURUZA

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### Sažetak

U odnosu na druge žitarice, zrno kukuruza sadrži visok nivo karotenoida i tokoferola. Od svih karotenoida  $\beta$ -karoten ima najveću aktivnost i smatra se najznačajnim u biofortifikacijskim programima oplemenjivanja žitarica. Ovo istraživanje je imalo za cilj da utvrdi razlike u morfološkim svojstvima, sadržaju tokoferola ( $\alpha$ -,  $\beta+\gamma$ -,  $\delta$ -),  $\beta$ -karotena, luteina i zeaksantina kod lokalnih i introdukovanih populacija kukuruza iz banke gena Instituta za kukuruz "ZemunPolje".

Sadržaj mikronutrienata je utvrđen primenom HPLC metode. Koeficijent varijacije za morfološka svojstva je bio manji od 10%, osim za prinos zrna po biljci ( $C_v=18,6\%$ ). Sadržaj  $\alpha$ -tokoferola je bio u rasponu od 1,04-8,42  $\mu\text{g g}^{-1}$  suve mase, a  $\beta$ -karotena od 0,26 to 7,95  $\mu\text{g g}^{-1}$  suve mase.  $\delta$ -tokoferol je bio u značajnoj korelaciji sa brojem zrna u redu ( $r=0,700^{***}$ ), dok je  $\beta+\gamma$ -tokoferol bio u značajnoj negativnoj korelaciji sa visinom biljke i klipa ( $r=-0,601^{***}$ ;  $r=-0,591^{**}$ ). Korelacije između morfoloških svojstava i  $\alpha$ -tokoferola bile su slabe i nesignifikantne.

Boja zrna je bila u značajnoj korelaciji sa sadržajem zeaksantina ( $r=0,590^{***}$ ) i  $\beta$ -karotena ( $r=0,398^*$ ). Za potrebe biofortifikacije "pool" od 11 populacija sa povećanim sadržajem i  $\beta$ -karotena i  $\alpha$ -tokoferola biće formiran na osnovu dobijenih rezultata.

**Ključne reči:**  $\beta$ -karoten, zrno, tokoferol, populacije, *Zea mays* L.

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