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MICRONUTRITIENT VARIABILITY IN MAIZE INBRED LINES

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ABSTRACT

Development of micronutrienten riched staple plant foods through plant breeding holds promise for sustainable and cost-effective food-based solutions to combat micronutrient deficiencies. The first step in this process is screening aviable germplasm for micronutritient content, so the aim of this study was determination of carotenoids and tocopherols content in set of maize inbred lines. Carotenoids (lutein, zeaxanthin and *β*-carotene) and tocopherols (α, β+γ and δ) content in 101 maize inbred lines with different kernel type (37 orange, 29 yellow, 4 white,19 sweetcorn and 12 popcorn) were determined by HPLC-DAD. The mean values of L+Z, β-carotene, α-tocopherol, β+γ tocopherol, and δ-tocopherol, were 31.34, 8.72, 10.22, 49.17 and 1.81 µg/ g, respectively. Content of α -tocopherol was in the range from 2.22 to 38.14 µg/g and β+γ tocopherols from 12.10 to 105.52 µg/g, β-carotene 1.20 to 39.37 µg/g and lutein+zeaxanthin 11.28 to 69.31 µg/g. White maize lacked carotenoids in the endosperm due to the presence of recessive genes. The highest value of β-carotene had inbred line H, L+Z inbred W-4, γ-tocopherols KRW 803-3-1-2-1 and α –tocopherol P21. Orange kernel inbred lines had the highest value of L+Z and β-carotene, yellow kernel inbred lines α –tocopherol, whereas sweetcorn inbreds had the highest value of γ-tocopherols. The genetic background undoubtedly influences chemical quality and line with high content of particularly micronutritients may be used in breeding program to improve nutritional value.

Keywords: *biofortification, carotinoids, maize, tocopherols.*

INTRODUCTION

Maize as one of the most widely cultivated cereals in the world have several types of kernal, with colours such as orange, yellow, red, black, and blue. Pigmented maize has received increased attention from a nutraceutical perspective because it contains several bioactive phytochemicals such as carotenoids, tocopherols, phytic acid and phenolic compounds (Bacchetti *et al.*, 2013). Generally, maize exhibits considerable natural variation for kernel carotenoids. The carotenoids present in maize grain are classified into carotenes (*β*-carotene, *α*-carotene) and xanthophylls (lutein, zeaxanthin and *β*-criptoxanthin), with higher concentrations of lutein and zeaxanthin compared to other carotenoids.

In plants, carotenoids prevent chlorophyll from photo-oxidative damage and protect membranes from lipid peroxidation, serve as precursors to abscisic acid (ABA), which regulates plant growth, embryo development, dormancy, and stress responses (Johnson et al. 2007). Carotenoids also have significant nutritional value for humans by providing precursor molecules required for the synthesis of vitamin A. The efforts thus directed to increase provitamin A and non-provitamin A carotenoids in maize will have positive impact on the health and well-being of humans, especially women and children. Tiwari et al. (2012) and Sivaranjani et al. (2013) observed positive correlation between kernel colour and total carotenoids but kernel colour is an unpredictable indicator for β-carotene so visual selection based on kernel colour will mislead in selecting provitamin A-rich genotypes (Harjes et al. 2008). Intensive efforts are currently being undertaken to develop maize inbreds enriched with carotenoids especially provitamin A (Babu *et al.* 2013; Frano *et al*. 2014).

Tocopherols (*α*-, *β*-, *γ*-, *δ*-), the subgroup of vitamin E, are biologically active compounds. There is natural variation among different maize inbred lines for levels of tocopherols. The two predominant isomers present in maize grain are gamma-tocopherol and alpha-tocopherol. Alpha-tocopherol is considered more desirable for human and animal consumption because it has higher biological activity than gamma-tocopherol. Most maize inbred lines naturally have much more gamma-tocopherol than alpha-tocopherol. Therefore a breeding goal is to increase levels of alpha-tocopherol relative to gamma-tocopherol. However, recent research suggests that gamma-tocopherol and compounds metabolized from it have properties important to human health that are unique from properties of alpha-tocopherol. Therefore it may be desirable to not only increase levels of alpha-tocopherol in maize grain, but also levels of gamma-tocopherol.

MATERIAL AND METODS

A diverse panel of 101 inbreds having different kernal type and color was selected and grown at Zemun Polje in 2017. Of them, 70 are inbreds with standard kernal type: 37 with orange color (1-37), 29 with yelow kernal (38-66), 4 white inbred lines (67-70), 19 sweetcorn (71-89) and 12 popcorn (90-101) inbred lines. Maize seed were milled (Perten 120, Sweden) into flour (particle size < 500 μm), in order to obtain greater surface contact and stored at -20˚C prior to analysis.

Carotenoids were extracted according to the slightly modificated method proposed by Rivera *et al* .(2012). Briefly, 0.15 g of the grain maize sample was extracted twice with 5 mL of mixture methanol-ethyl actetate (6:4, v/v) for 30 min at ambient room. Collected extracts after centrifugation (3000 rpm, for 5 min) were evaporated under the stream of nitrogen to the dryness and redissolved in 1mL of the mobile phase. Afterwards, extracts were filtered through syringe filter to be analyzed by Dionex UltiMate 3000 liquid chromatography system (Thermo Scientific,Germany) fitted with photodiode array detector (DAD–3000) analytical column, Acclaim Polar Advantage II, C18 (150 × 4.6 mm, 3 µm). Mixture of methanol-acetonitrile, (90:10, v/v) was used as mobile phase. The flow rate was 1 mL/min, temperature of column was set at 25 ºC and the injection volume was 50 µL. Chromatograms were generated at 450 nm and 470 nm. Tested carotenoids were identified and quantified by comparing characteristic retention time of appropriate standards. Carotenoids content is expressed as micrograms per gram of dry matter.

Tocopherols (α-, γ+ *β*- and δ) were extracted by method proposed by Gliszczyńska-Świgło *et al* (2007) with minor modification. Approximately 0.2 g of sample was mixed with 2-propanol (4 mL) and homogenised for 30 min at room temperature. The extracts were then centrifuged at 3000 rpm for 5 min, filtered through a 0.45 µm membrane filter and a aliquot of clear supernatant was directly injected into the Dionex UltiMate 3000 liquid chromatography system (Thermo Scientific, Germany) equipped with fluorescence detector (FLD–3100). Chromatographic separation of tocopherols was accomplished on analytical column, Acclaim Polar Advantage II, C18 (150 × 4.6 mm, 3 µm) operated at 30ºC. Mixture of acetonitrile and methanol (1:1, v/v) was used as a mobile phase at flow rate of 1 mL/min. Injection volume was 5µL, while the wavelengths for excitation and emission were maintained at 290 nm and 325 nm, respectively. Tocopherols were identified and quantified comparing characteristic retention time of corresponding standards. The tocopherols content is expressed as micrograms per gram dry matter.

RESULTS AND DISCUSSION

The study revealed wide genetic variation for the kernel carotenoids lutein+ zeaxanthin and β-carotene in maize inbred lines with different kernal type and color. The mean values of L+Z and β-carotene were 31.34 and 8.72 µg/g, respectively. Content of β-carotene was in the range from 1.20 to 39.37 µg/g. The inbreds H (39.37 µg/g), W-4 (32.47 µg/g) and Fd-96 with orange kernel were found promising for β-carotene. Content of lutein+zeaxantin was in the range from 11.28 to 88.06 µg/g. The inbreds W-4 (88.06 µg/g) and TVA912 (69.31 µg/g) with orange kernal were identified with high lutein+zeaxantin. Orange kernel inbred lines had the highest mean value of L+Z and β-carotene (35.62 and 14.81 µg/g), followed by popcorn inbred lines with orange to dark yellow kernel (29.67 and 6.26 µg/ g). The carotenoid content in white kernel inbred lines had not be detected. White maize lacks carotenoids in the endosperm due to the presence of recessive *y1* or *phytoene synthase (psy1)*, the key gene that controls the first step in the carotenoid biosynthesis pathway, whereas, the yellow/orange kernel maize with dominant *Y1* contains α-carotene and β-carotene and zeaxanthin and lutein (Buckner *et al*. 1990).

Table 1. Content of carotenoides in 101 maize inbred lines

No	L+Z	β-karoten	No	L+Z	β-karoten	No	L+Z	β-karoten
1	36,08±0,03	3,38±0,00	35	22,89±0,09	15,07±0,06	69	ND	ND
2	24,84±0,38	3,20±0,03	36	36,22±0,28	8,45±0,03	70	ND	ND
3	45,40±0,27	39,37±0,24	37	69,31±0,51	10,56±0,08	71	28,67±0,32	2,66±0,03
4	46,17±0,61	16,87± 0,22	38	22,19±0,07	4,79±0,02	72	28,49±0,18	8,59±0,05
5	49,00±0,77	22,08±0,35	39	29,59±0,28	8,72±0,08	73	25,54±0,15	3,71±0,01
6	43,45±0,83	21,12±0,41	40	36,52±0,21	16,29±0,09	74	29,03±0,15	5,21±0,03
7	23,64±0,15	30,13±0,19	41	19,70±0,12	2,23±0,01	75	36,29±0,31	3,46±0,01
8	37,22±0,14	7,12±0,03	42	24,22±0,12	5,65±0,03	76	30,08±0,22	2,01±0,01
9	17,81±0,24	11,41±0,15	43	28,31±0,46	9,14±0,15	77	25,19±0,23	2,08±0,02
10	20,94±0,27	15,34±0,20	44	26,87±0,14	5,39±0,03	78	21,21±0,29	3,66±0,05
11	36,23±0,49	22,72±0,31	45	51,76±0,49	8,51±0,08	79	21,18±0,09	3,23±0,01
12	20,35±0,27	14,76±0,20	46	35,67±0,36	4,40±0,04	80	28,54±0,21	7,21±0,05
13	33,82±0,15	2,72±0,06	47	24,75±0,16	2,50±0,02	81	26,28±0,13	1,83±0,01
14	26,75±0,08	14,51±0,05	48	42,37±0,34	4,94±0,04	82	28,24±0,31	2,42±0,03
15	22,62±0,11	5,46±0,03	49	22,58±0,17	3,70±0,03	83	20,60±0,33	3,46±0,02
16	40,91±0,27	12,66±0,08	50	33,80±0,41	5,17±0,06	84	31,10±0,23	2,84±0,01
17	26,63±0,08	11,01±0,03	51	53,14±0,36	7,58±0,05	85	28,14±0,27	4,59±0,04
18	49,52±0,81	6,47±0,11	52	34,13±0,29	10,49±0,09	86	35,99±0,18	4,01±0,02
19	88,36±1,08	32,47±0,40	53	31,47±0,35	5,48±0,06	87	25,58±0,15	5,82±0,03
20	40,78±0,22	10,32±0,05	54	19,59±0,14	1,91±0,01	88	24,79±0,27	2,40±0,03
21	33,05±0,32	6,67±0,07	55	28,58±0,25	7,97±0,07	89	37,96±0,48	3,31±0,04
22	31,78±0,19	15,70±0,09	56	21,06±0,38	1,41±0,03	90	24,17±0,14	5,47±0,03
23	29,14±0,27	6,75±0,06	57	33,25±0,35	9,90±0,10	91	17,94±0,20	8,49±0,10
24	29,16±0,18	16,91±0,11	58	11,28±0,16	1,70±0,02	92	21,22±0,07	4,93±0,02
25	34,23±0,20	14,36±0,09	59	16,04±0,06	1,95±0,01	93	25,67±0,11	4,92±0,02
26	23,78±0,07	25,45±0,07	60	23,70±0,10	2,48±0,01	94	27,12±0,27	4,55±0,04
27	29,83±0,45	9,73±0,15	61	30,06±0,24	3,10±0,03	95	33,90±0,13	9,88±0,04
28	49,86±0,77	26,16±0,40	62	31,24±0,18	3,34±0,02	96	24,03±0,14	4,81±0,03
29	39,05±0,11	8,65±0,02	63	28,90±0,43	3,28±0,05	97	30,78±0,41	4,12±0,05
30	17,56±0,18	10,13±0,10	64	17,13±0,16	1,20±0,01	98	38,12±0,39	10,14±0,10
31	27,56±0,25	16,69±0,15	65	29,29±0,12	5,56±0,02	99	39,69±0,49	11,67±0,14
32	33,30±0,08	8,79±0,02	66	25,69±0,21	1,30±0,01	100	31,07 ±0,18	2,33±0,01
33	31,60±0,06	25,05±0,04	67	ND	ND	101	42,36±0,27	4,04±0,03
34	39,21 ±0,14	14,67±0,05	68	ND	ND			

The genetic variation for various carotenoids among maize inbreds showed zeaxanthin and lutein were the most predominant carotenoids. Vignesh *et al.* (2012) reported the same low range of kernel β-carotene as well as Chander *et al.* (2008) observed a similar trend of variation while evaluating a set of Chinese germplasm, and found less provitamin A concentration and more of lutein and zeaxanthin. The carotenes (α- and β-carotene) are the intermediates in the carotenoid biosynthesis pathway, leading to the more synthesis of non-provitamin A (lutein and zeaxanthin), which is why maize kernels generally have limited provitamin A despite being rich in other carotenoids (Vallabhaneni *et al.* 2009).

Table 2. Content of tocopherols in 101 maize inbred lines

No	δ	β+γ	α	No	δ	β+γ	α
1	2,04±0,01	28,55±0,13	10,59±0,05	52	0,95±0,01	53,30±0,39	2,68±0,02
2	1,98±0,02	35,73±0,28	14,15±0,11	53	1,85±0,01	30,90±0,23	17,91±0,13
3	1,08±0,01	25,18±0,30	24,04±0,29	54	1,04±0,03	69,69±0,32	6,33±0,03
4	1,68±0,05	79,01±0,56	4,26±0,03	55	1,21±0,01	35,81±0,11	9,76±0,03
5	2,95±0,03	51,61±0,60	8,16±0,09	56	0,81±0,00	23,33±0,10	19,12±0,08
6	2,53±0,06	105,52±1,37	5,94±0,08	57	2,02±0,01	41,88±0,27	19,78±0,13
7	1,80±0,01	44,54±0,20	7,23±0,03	58	1,62±0,01	28,89±0,14	13,62±0,07
8	0,74±0,01	18,25±0,21	9,73±0,11	59	1,46±0,01	46,60±0,20	14,24±0,06
9	1,29±0,03	37,51±0,33	4,67±0,04	60	1,60±0,01	7 2,18±0,41	6,80±0,09
10	1,38±0,01	16,31±0,08	2,90±0,01	61	0,66±0,01	30,31±0,34	16,15±0,18
11	4,39±0,03	53,09±0,35	10,84±0,07	62	0,92±0,01	44,11±0,34	15,56±0,12
12	1,18±0,01	29,08±0,16	8,66±0,05	63	1,26±0,01	45,00±0,47	13,82±0,15
13	2,08±0,02	58,89±0,68	4,72±0,05	64	0,49±0,00	27,16±0,13	4,41±0,07
14	2,99±0,02	81,69±0,66	7,60±0,06	65	1,36±0,00	23,11±0,05	9,81±0,02
15	1,15±0,01	33,35±0,36	12,16±0,13	66	0,49±0,00	16,19±0,14	11,16±0,09
16	1,40±0,01	39,05±0,23	3,38±0,01	67	1,45±0,04	72,13±0,66	3,72±0,03
17	1,73±0,00	63,77±0,18	18,00±0,05	68	1,22±0,01	33,89±0,16	13,35±0,06
18	2,77±0,03	62,40±0,64	4,41±0,04	69	0,61±0,00	35,74±0,13	18,70±0,07
19	2,29±0,02	77,46±0,54	9,85±0,07	70	1,20±0,00	43,97±0,11	12,37±0,03
20	0,77±0,01	41,42±0,31	9,44±0,07	71	1,65±0,03	50,09±0,81	15,60±0,25
21	5,48±0,05	105,02±0,92	9,82±0,09	72	1,80±0,01	53,88±0,21	13,73±0,05
22	1,39±0,01	28,16±0,28	3,74±0,02	73	0,94±0,02	69,26±0,39	3,22±0,02
23	2,29±0,02	41,24±0,38	38,14±0,35	74	2,14±0,03	57,05±0,78	6,25±0,09
24	2,74±0,03	71,30±0,80	10,62±0,12	75	2,62±0,05	77,11±1,43	7,66±0,14
25	0,50±0,01	14,17±0,17	7,40±0,09	76	2,17±0,01	52,95±0,21	14,99±0,06
26	0,79±0,00	19,20±0,11	12,93±0,08	77	3,66±0,02	75,26±034	10,68±0,05
27	0,85±0,01	27,49±0,17	2,35±00,01	78	3,51±0,02	86,12±0,38	16,58±0,11
28	2,01±0,01	62,17±0,24	10,05±0,04	79	2,38±0,02	83,87±0,30	8,83±0,03
29	4,27±0,03	71,70±0,53	21,73±0,16	80	2,52±0,02	63,32±0,38	8,41±0,05
30	1,23±0,03	57,28±0,50	3,87±0,03	81	4,66±0,11	99,26±0,24	12,28±0,28
31	2,77±0,02	63,77±0,40	11,31±0,07	82	2,70±0,28	59,99±0,28	12,96±0,36
32	0,46±0,01	42,63±0,34	2,67±0,02	83	4,01±0,02	101,32±0,54	17,00±0,09
33	6,56±0,06	56,60±0,50	12,37±0,11	84	1,16±0,09	88,38±0,15	3,97±0,05
34	1,74±0,01	20,11±0,17	7,86±0,07	85	1,69±0,08	82,99±0,48	9,20±0,16
35	0,96±0,01	12,10±0,10	7,84±0,06	86	2,14±0,01	59,79±0,15	13,57±0,03
36	3,83±0,02	64,59±0,34	7,81±0,04	87	3,01±0,02	78,67±0,61	17,79±0,14
37	1,90±0,01	45,64±0,18	17,26±0,07	88	2,09±0,02	53,61±0,47	9,40±0,08
38	1,82±0,01	47,73±0,30	3,33±0,02	89	1,29±0,05	64,47±0,95	10,70±0,16
39	1,21±0,01	21,76±0,24	13,24±0,15	90	2,31±0,02	36,33±0,37	6,36±0,06
40	1,25±0,06	73,15±0,72	6,73±0,02	91	1,45±0,06	40,27±0,30	5,20±0,01
41	0,77±0,03	55,25±0,47	3,72±0,01	92	1,73±0,07	39,19±0,56	4,14±0,02
42	1,69±0,01	47,63±0,40	8,77±0,07	93	2,03±0,02	38,08±0,16	5,60±0,02
43	0,83±0,05	61,51±0,82	2,22±0,03	94	0,98±0,01	35,15±0,12	3,50±0,01
44	4,53±0,04	40,40±0,37	10,80±0,10	95	1,59±0,02	48,23±0,32	3,39±0,01
45	0,70±0,00	24,27±0,05	12,99±0,03	96	0,70±0,04	39,73±0,39	3,12±0,01
46	0,59±0,00	21,89±0,18	20,52±0,17	97	1,27±0,02	29,19±0,21	3,65±0,02
47	1,20±0,01	31,72±0,20	20,24±0,13	98	1,57±0,05	48,22±0,44	5,76±0,03
48	0,79±0,00	20,20±0,08	12,12±0,05	99	1,19±0,01	45,11±0,17	4,53±0,01
49	1,43±0,01	36,01±0,24	3,10±0,01	100	0,71±0,03	41,09±0,39	3,75±0,02
50	1,74±0,01	33,82±0,14	13,67±0,06	101	1,13±0,02	60,45±0,19	4,49±0,01
51	1,18±0,01	32,34±0,20	16,66±0,11				

In breeding program for selection of high carotenes maze lines, Safawo *et a*l. (2010) have also determined high variation in β-carotene in maize grains. Vignesh *et al* (2015) found wide genetic variation for lutein (0.36–15.75 μg/g), zeaxanthin (0.25–22.76 μg/g), and β-carotene (0.07–17.41 μg/g) in 48 diverse maize inbreds. According to Chander *et al.* (2008) allelic variations and dosage effects may be responsible for the wide range of variability for carotenoids in yellow maize. Inbreds with high lutein and zeaxanthin identified in the study can be used in developing hybrids specifically for poultry industry and inbreds with high β-carotene (~15 μg/g) would be useful in developing provitamin A enriched hybrids to alleviate vitamin A deficiency.

In the reverse phase system, which was employed in this study, there is no possibility to separate *β*- and *γ*- tocopherol, so their content is expressed as their sum (Abidi, 2009, Gliszczyńska-Świgło *et al.*, 2004). The mean values of α-tocopherol, β+γ tocopherol, and δ-tocopherol, were 10.22, 49.17 and 1.81 μg/g, respectively. Sweetcorn inbred lines have the highest average value of *β+γ* tocopherols (59.61 μg/g) followed by orange kernal inbred lines (48.26 μg/g), popcorn (41.75 μg/g), white kernal inbred lines (40.38 μg/g) and yellow kernal inbred lines (39.18 μg/g). That are in accordinace with results of our previously study of tocopherols content in different set of inbred lines (Drinic *et al*., 2017) as sweetcorn inbred lines had higher *β+γ* tocopherols content than standard kernel type inbred lines. Average values of *α* tocopherol in orange, yelow, white inbred lines, sweetcorn and popcorn was 9.96, 12.04, 12.03, 8.65 and 4.48μg/g, respectively. The highest value of γ-tocopherols have inbred line KRW 803-3-1-2-1 (105.52 μg/g) and α –tocopherol TVA973 (38.14 μg/g). The inbred line W-4 with orange kernels had a high level of *β*-carotene and lutein+zeaxantin, as well as moderate content of tocopherols. Orange kernel inbred lines had the highest value of L+Z and β-carotene, yelow kernel inbred lines α –tocopherol, whereas sweetcorn inbreds had the highest value of γ-tocopherols. The genetic background undoubtedly influences chemical quality and line with high content of particularly micronutritients may be used in breeding program to improve nutritional value.

CONCLUSIONS

Carotenoides and tocopherols are compounds present in maize kernal that provide health and economic benefits, which potentially could be captured by both producers and consumers to add value to the grain. So, increased levels of caritenoids and tocopherols in maize grain, because of their antioxidant activity, should increase the nutritive value of maize. The great natural variation for carotenoids and tocopherolos are presented in maize inbred lines with different kernal type (normal, sweetcorn, popcorn) and kernal color (white, yellow, orange). The inbred line W-4 with orange kernels had a highest content of lutein+zeaxantin and high β-carotene, as well as moderate content of tocopherols so could be use in breeding program for parallel improvement of both carotenoids and tocopherols.

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