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VARIJABILITY OF TOCOPHEROL AND B-CAROTINE CONTENTS IN MAIZE GENOTYPES

Snežana Mladenović Drinić, Vesna Dragičević, Slađana Žilić, Zorica Basić, Dragan Kovačević
[1]Maize Research Institute Zemun Polje, Belgrade, Serbia, [2]VMA, Belgrade, Serbia

Abstract

Maize displays considerable natural variation for macronutritiens and micronutritiens, including vitamin A precursor's β-carotene and tocopherols. Among staple crops, maize has a high amount of carotenoids and tocopherols, which have a number of beneficial effects on human health. Tocopherols has important role in food production preventing lipids from oxidation. The objectives of this study were to estimate tocopherol and β-carotine contents as well protein, oil and starch content in 17 maize genotypes. A high amount of variation for β-carotine and tocopherol content is present among genotypes as well as for different ratio of α- and γ-tocopherols. Content of α -tocopherol was in the range from 3.21 to 16.52 µg/g and γ-tocopherols from 2.99 to 17.11 µg/g. Increased levels of carotenoids and tocopherols in corn grain, because of their antioxidant activity, should increase the nutritive value of corn.

Key words: *maize, macronutrients, tocopherols, β carotene*

1. INTRODUCTION

Maize is one of the most important crops due to its high productivity and its multiple uses as a food source for humans, livestock feed and as raw material in various industries. The three major components of a grain of maize are starch, protein, and oil. Average content of proteins, oil and starch in maize seed is 9%, 4%, 73%, respectively (Balconi *et al.*, 2007). The genetic background undoubtedly influence chemical quality of maize hybrids and may be modified in profit of the chemical constitution and so achieve new germplasm with excellent attributes related to industrialization and nutritional value. Development of maize with unique grain quality traits, however, has not received the same emphasis in genetics, breeding, and economics as higher yield and agronomic performance (Hallauer, 2001; Scott *et al*., 2006). Some breeding programs are routing the specific traits for different usage of maize grain, such as increasing of oil, protein or starch content in grain (Saleem *et al.*, 2008; Rosulj *et al.*, 2002, Pollak and Scott, 2005, Harrelson *et al.*, 2008, Idikut *et al.*, 2009). In addition carotenoid and tocopherol content has been a focus of modern agriculture and several QTLs for tocopherol content has been mapped in different plant species.

Carotenoids (provitamin A) and tocopherols (vitamin E) are lipid soluble antioxidants associated with decreased risk of several degeneative diseases. Both vitamins occur naturally in maize grain. Carotenoids are located in endosperm and tocopherols, a component of oil, in the germ (Weber, 1987; Grams *et al*., 1970). Several studies have shown significant differences among corn inbreds for carotenoid and tocopherol levels (Blessin *et al*., 1963; Quackenbush *et al*., 1963; Egesel, 1997).

Two classes of carotenoid pigments are carotenes and xanthophylls, which are responsible for the yellow and orange color of corn endosperm. Among the major food grains, only yellow corn contains significant levels of β carotene, a source of vitamin A (Buckner *et al.*, 1990). Carotenoid compounds are derivatives of a well described secondary metabolic pathway in plants, participating in a diverse array of physiological functions, and are nutritionally valued vitamin precursors in the human diet.

Vitamin E is the common name that describes eight naturally occurring compounds having tocopherol activity (Van Eenennaam *et al.* 2003). The eight compounds are lipid-soluble antioxidants with two distinct groups, tocopherols and tocotrienols. The two groups differ in the saturation of the side chain and vary in the number and location of methyl groups, and are classified according to the location of the methyl group: α-tocopherol (αT), β-tocopherol (βT), δ-tocopherol (δT), γ-tocopherol (γT), α-tocotrienol, β- tocotrienol, δ-tocotrienol and γ-tocotrienol.

Vitamin E antioxidant activity order is α –Tocopherol > β – Tocopherol > γ – Tocopherol > δ – Tocopherol . α-tocopherol is the major vitamin E in nearly all green plants, in non-green plant parts, such as seeds and fruits, γ-tocopherol is preferentially found instead (Matea *et al*., 2008).

Many studies showed vitamin E has a number of vital functions in plants, including the protection of chloroplasts from photo oxidative damage (Munne-Bosch and Alegre 2002). Moreover, human nutritional studies have suggested that vitamin E might play an important role in enhancing the function of the immune system (Adachi *et al*. 1997), and the treatment or prevention of a number of serious diseases including cardiovascular disease, Alzheimer's disease, neurological disorders, cancer, cataracts, inflammatory diseases and age-related macular degeneration (Bramley *et al.* 2000). Food and nutrition guidelines recommend 15 mg/day of vitamin E for both adults and teenagers (Institute of medicine, 2000).

Our understanding of the genetic basis underlying trait variation has expanded recently as maize genomics, model and crop species physiology, biotechnology, and statistical methods have been development (www.maizesequence.org). Varieties with improved nutritional qualities including oil composition, essential amino acid content, and starch type have been successfully developed, leading to many products that are commercially available today (Graham *et al*., 2001). Enhanced grain micronutrient content for both vitamins and minerals is rapidly emerging as the next suite of seed quality traits to be improved by breeding and biotechnology.

The aim of this study was comparison of protein, oil, starch, β carotene, and tocopherol content of 8 maize hybrids and their parental lines.

2. MATERIAL AND METHOD

A set of 8 hybrids of FAO maturity groups from 300 to 600 and their parental inbred lines was used. Hybrid~~s~~ ZP341 (L1×L5) belonged to FAO maturity group 300; ZP 427 (L9×L2) belonged to FAO maturity group 400, ZP555 (L3×L5) and ZP560 (L7×L6) belonged to FAO maturity group 500, ZP600 (L7×L8); ZP606 (L3×L8), ZP666 (L3×L6) and ZP684 (L8×L4) belonged to FAO maturity group 600.

An experiment set up as randomized block design with three replications at experimental field of MRI. The protein, oil and starch content were determined by near-infrared reflectance spectroscopy NIR using Infratec 1241 grain analyzer (Foss Tecator, Sweden) and expressed in a percentage of dry mater.

The content of tocopherols was determined by the HPLC method. An n-hexane extraction was applied. The maize flour sample (1 g) was mixed with 10 mL of n-hexane and the mixture was rigorously shaken at 4 ^{o}C for 30 min. After centrifugation at 7,000 *g* for 15 min, the upper layer was separated and evaporated under N_2. The dried sample was then redissolved in 5 mL of methanol, vortexed, centrifuged at 5,000 *g* for 10 min, and the clean upper layer was collected. A HPLC system with the Waters M600 E pump, thermostat and Rheodyne 7125 injector was used. The separation of tocopherols was performed on the Nucleosil 50-5 C18 column (250 × 4 mm, i.d., 5 µm) at flow rate of 1.0 mL min^{-1}. The mobile phase consisted of 95% methanol. The detection was performed with the Shimadzu RF-535 fluorescence detector at an excitation wavelength of 295 nm and an emission wavelength of 330 nm. Identified peaks were confirmed and quantified by data acquisition and spectral evaluation using the "Clarify" chromatographic software. The content of tocopherols is expressed as µg per g of d.m. Β-carotene was determined according to AACC (1995) procedure after extraction with saturated butanol.

Heterosis was determined as follows:

Mid-parent heterosis (%) = ((F1- MP)/MP) × 100

Best-parent hetrosis (%) = ((F1- BP)/BP) × 100

Where

F1 =Mean of F1 hybrid for a specific trait

MP=Mean of the two parents in a cross for a specific trait

BP= Mean of the best parent in a cross for a specific trait

3. RESULTS AND DISSCUSSION

Maize as the other cereal crops is relatively poor in grain protein content as usually varies from 8.0 to 11.0% according to FAO reports. In general, maize protein content varies according to genotype (Lorenz *et al.*, 2007, Drinic *et al.*, 2009, Stevanović *et al.* 2012). The range of protein content was wider for parental lines than for hybrids. Hybrids has protein content between 9.81% (ZP684) to 11.42 (ZP555), average 10.51% and parental lines between 9.85% (ZPL5) to 12.84 (ZPL2); average 10.58, table 1. Obtained results is agreement with findings of Corcuera *et al.* (2004), that protein content of 72 maize hybrids varied form 8,2 to 12,6 %, average

10,5%. Two inbreeds ZPL2 and ZPL3 indicated high protein content (12.84%, 10.8, respectively). On the other hand, i nbred ZPL5 and ZPL 6 showed slightly low protein content (9.85%, 9.86%, respectively). Two crosses have protein content higher than average, ZPL427 (11.38%), and ZPL 555 (13.35%), and the other six crosses showed slightly low protein content. These results are in agreement with those obtained by Seiam and Khalifa (2008), Olivera *et al.* (2006), Idikut *et al* (2009) and Stevanovic *et al*. (2012). Hybrid with the highest protein content was combination of one inbred line with high protein content and one with the lowest protein content among inbred lines. ZP684, as combination of one line with high protein content and one with middle-low have the lowest protein content.

Table 1. Protein, oil and starch content

Genotypes	Protein %	Oil %	Starch %
ZP341	10.11	4.68	71.06
ZP427	11.38	4.49	69.98
ZP555	11.42	4.00	70.68
ZP560	10.45	5.00	71.15
ZP600	10.16	4.14	72.45
ZP606	10.14	3.85	72.32
ZP666	10.49	4.60	71.31
ZP684	9.81	3.86	72.34
ZPL1	10.07	3.35	68.98
ZPL2	12.84	3.45	68.59
ZPL3	10.98	3.38	71.82
ZPL4	10.79	4.00	71.78
ZPL5	9.85	4.49	71.29
ZPL6	9.95	4.47	70.65
ZPL7	10.65	4.18	71.88
ZPL8	10.05	3.56	71.68
ZPL9	10.00	4.01	71.16

Oil content in maize inbred line, presented in Table 1, were significantly different from each other and ranged from 3.35% to 4.49%, average 3.88%. The lowest values of oil were found in the two inbreeds ZPL1 (3.35%) and ZPL3 (3.38%), while the highest values were observed in inbred ZPL5 (4.49%), and ZPL6 (4.47%). Regarding the hybrids, oil content varied from 3.85 (ZP606) to 5.00 (ZP560), average 4.33%. Four hybrids had higer oil content than average. Mittelmann *et al*. (2006) found that means of 10 maize genotypes and their hybrids varied from 4.22% to 4.94% for oil content. Corcuera *et al* (2004), obtained little higher values for oil content in maize hybrids, from 3,52 to 6,41%, average 5,24%. The hybrid with the highest oil content, ZP560, was combination of one inbred line with high oil content and one with middle-high. ZP606, combination of two inbred lines with low oil content has the lowest one.

The starch is the largest single components in maize grain and the primary energy source. Average starch content was higher for hybrids than parental lines, 71.41% and 70.87%, respectively. The starch content range from 69.98 (ZP341) to 72.45% (ZP600) for hybrids, and from 68.59 (ZPL2) to 71.88% (ZPL7), for parental lines. This result is in agreement with findings of Drinic *et al.* 2009, Stevanovic *et al*, 2012. Three ZP hybrids had starch content higher than average. ZP600, combination of one inbred line with high starch content and one with middle –high, has the highest starch content. The lowest one has hybrid ZP427, combination of one inbred lines with middle–low starch content and inbred line with the lowest starch content among parental lines. Hybrid ZP427 with the lowest starch content has high protein and middle-high oil content. Hybrid ZP555 with the highest protein content has low oil and starch content. ZP684 have the lowest protein content as well as low oil content but the second high starch content. Also, ZP606 with the lowest oil content have low protein content and high starch content.

Obtained values for protein, oil and starch are typically for maize genotypes that were not specifically selected for those traits (Pollak and Scott, 2005; Harrelson *et al*., 2008; Idikut *et al.*, 2009).

Among hybrids four has positive and four negative values of mid-parent heterosis for protein content, but for better-parent heterosis six hybrids has negative value (Table 2). These results are in agreement with results of Abou-Deif *et al* (2012) who reported that six hybrids have positive heterosis over the best parent, and nine negative heterosis below the best-parent and mid-parents values. The highest positive heterosis was obtained in hybrid ZP555 with the highest protein content. All studied crosses showed positive heterotic values for oil content comparing to the mid parent, ranged between 1.52 to 20.37%, and three of them manifested significant heterosis, i.e. ZP427 (20.37%), ZP341 (19.39%) and ZP666 (17.35%). Comparing to the better parent three crosses gave negative heterotic values. Renuka *et al.* (2008) observed high heterosis for oil content over the best parent value. On the other hand, Mittelmann *et al* (2006) evaluated oil content of 10 maize genotypes and showed mean heterosis -0.14, indicating that there is dominance for low oil content. Six hybrids have positive value of mid parent heterosis for starch content and five has negative value for better parent heterosis.

Table 2. Midparent and better parent heterosis for protein, oil and starch content

	Protein content		Oil content		Starch content	
Genotypes	MPH	BPH	MPH	BPH	MPH	BPH
ZP341	1.5	0.40	19.39	3.56	1.37	-0.32
ZP427	-0.35	-11.37	20.37	11.97	0.14	-1.66
ZP555	10.56	5.10	1.52	-10.91	-1.16	-1.59
ZP560	1.47	-1.87	15.47	11.86	-0.14	-1.01
ZP600	-1.84	-0.43	1.80	-0.96	0.94	0.79
ZP606	-3.61	-7.65	10.95	8.14	0.79	0.70
ZP666	0.19	-4.46	17.35	2.90	0.09	-0.71
ZP684	-5.85	-9.08	2.12	-3.5	0.85	0.78

Average content of α tocopherol was higher in parental lines (1049.28 μg/kg^{-1}) than hybrids (966.18 μg/kg^{-1}) but hybrids have higher content of γ tocopherol (956.59 μg/kg^{-1}) than parental lines (692.02 μg/kg^{-1}). Two of eight hybrid and all inbred lines except ZPL5 has content of δ tocopherol <20 μg/kg^{-1}. Content of α tocopherol varied from 556.33 to 1283.14 μg/kg^{-1} for hybrids and from 321.71 μg/kg^{-1} to 1270.2 μg/kg^{-1} in parental lines. Hybrid ZP666 with the highest α tocopherol content is combination of inbred line with high and one with middle-high α tocopherol content. The highest α tocopherol content have inbred line ZPL6 (1652.85 μg/kg^{-1}) and the lowest ZPL 1 (321.71 μg/kg^{-1}). γ tocopherol content range from 597.30 μg/kg^{-1} to 1392.75 μg/kg^{-1} for hybrids and from 299.63 μg/kg^{-1} to 1415.31 μg/kg^{-1} for parental lines. Inbred line with the highest α tocopherol content has the lowest γ tocopherol content. Hybrid ZP684 has the lowest α tocopherol content but the highest γ and δ tocopherol content. Hybrids ZP606 and ZP666 with ZPL3 as common parent have high α tocopherol content and hybrids ZP600, ZP606 and ZP684 with ZPL8 as common parent have high γ tocopherol content. Two hybrids and five parental lines have highest α tocopherol content, three hybrids and four inbred lines have highest γ tocopherol content than average. Ratio of α- and γ-tocopherols range from 0.38 to 1.04 for hybrids and from 0.4 to 5.5 for parental lines (Table 3). Hybrids ZP427, ZP606 and ZP666 as well as inbred lines ZPL3, ZPL4, ZPL5, ZPL6, ZPL7, ZPL8 and ZPL9 have higher content of α tocopherol than γ tocopherol.

Table 3. Tocopherol and beta carotene content

Genotypes	α tocopherol $\mu g/kg^{-1}$	γ tocopherol $\mu g/kg^{-1}$	δ tocopherol $\mu g/kg^{-1}$	β carotene mg/kg	α / γ tocopherols ratio
ZP341	819.99	895.54	51.39	15.23	0.92
ZP427	810.06	778.30	70.18	21.90	1.04
ZP555	825.44	874.35	35.81	14.86	0.94
ZP560	569.72	597.30	<20	14.41	0.95
ZP600	950.33	1243.01	28.88	11.69	0.78
ZP606	1166.54	1042.79	55.54	19.07	1.12
ZP666	1283.14	828.70	<20	14.05	1.54
ZP684	556.33	1392.75	94.61	11.36	0.38
ZPL1	321.71	733.56	<20	16.71	0.44
ZPL2	565.14	1415.31	<20	14.94	0.40
ZPL3	1041.39	578.42	<20	16.88	1.81
ZPL4	1270.20	702.58	<20	18.61	1.80
ZPL5	1172.09	487.88	37.60	10.49	2.40
ZPL6	1652.85	299.63	<20	9.17	5.50
ZPL7	785.15	686.28	<20	8.69	1.14
ZPL8	1205.76	783.25	<20	17.74	1.54
ZPL9	1429.30	541.44	<20	18.17	2.64

Hybrids have average content of β carotene 15.32 mg/kg and parental lines 14.6 mg/kg. Hybrid ZP427 has the highest β carotene content (21.90 mg/kg) and hybrid ZP684 the lowest one (11.36 mg/kg). Inbred line ZPL 4 has the highest β carotene content (18.61 mg/kg) and ZPL7 the lowest one (8.69 mg/kg). 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.

In breeding for increased concentrations of carotenoids and tocopherols, it is important to know what effect male and female genotypes contribute to kernel content for these compounds when different genotypes are crossed. Egesel (2001) evaluated a group of orange and yellow endosperm hybrids and their parents for endosperm dosage effects. He found a dosage effect for the four carotenoids and total carotenoids in seeds of a group of reciprocal crosses among hybrids. Carotenoid concentrations of F2 seeds of these hybrids was closer to the female parent although the pollen parent did effect carotenoid levels. However, the female effect on carotenoid concentration was much larger than the male effect. This effect probably is the result of two doses of the female alleles in the endosperm and only one dose from the pollen parent. We also found that female parent have greater effect on β carotene content than male parent.

For α tocopherol 5 hybrid have negative heterosis according mid-parent and all for beter-parent heterosis (Table 4). Hybrids ZP560 and ZP684 have significant negative value. Only hybrid ZP427 has negative mid-parent heterosis for γ tocopherol as well as for beter-parent heterosis. Hybrid ZP560 also has negative better parent heterosis for γ tocopherol. Hybrids ZP666 and ZP684 has significant positive heterosis. Three hybrids for mid-parent and 5 hybrids for better parent heterosis have negative value for β carotene. Hybrids ZP560 and ZP427 have high positive heterosis.

Table 4. Midparent and better parent heterosis for tocopherols and beta carotene content

	α tocopherol		γ tocopherol		β carotene	
Genotypes	MPH	BPH	MPH	BPH	MPH	BPH
ZP341	9.06	-30.04	46.64	22.08	16.26	-8.85
ZP427	-18.74	-43.30	-37.54	-45.00	54.33	46.58
ZP555	-25.42	-29.57	64.00	51.16	8.56	-11.97
ZP560	-53.26	-65.53	21.16	-12.96	61.36	57.14
ZP600	7.33	-21.18	69.18	58.70	-11.50	-34.06
ZP606	3.83	-3.25	53.17	33.14	10.23	7.55
ZP666	-4.75	-22.37	88.77	43.27	7.91	-16.76
ZP684	-55.06	-56.20	87.37	71.43	-37.48	-38.96

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