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Response of some maize hybrids (*Zea mays* L.) for foliar application by polyisoprenic on bioactive components grown under middle Egyptian conditions

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Abstract

Grains of three maize hybrids grown in the middle Egypt zone were obtained and investigated for study the effect of spraying treatment with two β-carotene (βC) levels and natural carotenoids extract (NCE). The maize hybrids are yellow hybrid (No. 352) Yellow hybrid (No.178) and High Tec. White (No.2031). Results indicate that the most efficient solvent for carotenoids extraction is boiled methanol whereas the extract weight reached to be 0.913 and 0.9692 g/250 g dry weight grains followed by chloroform and ethanol. Application results of βC and NCE on crude lipids show that yellow hybrid (No. 352) recorded the highest levels followed by yellow hybrid (No. 178) and the lowest levels are recorded in High Tec. White (No. 2130). The concentrations with 7.5 and 15 µg/mL βC are better than NCE. It could be concluded that response of lipids in maize hybrids for foliar application are low. The yellow corn genotypes studied here are characterized with high levels of different isoforms of carotenes and xanthophylls and the results show that the most predominant carotene is β-carotene and the three predominant xanthophyll are zeaxanthin folowed by lutein and β-cryptoxanthin. Total carotenoids in untreated and treated samples ranged from 47.63 to 568.49 µg /g and the highest concentration was recorded with 15 ppm β-carotene as foliar spraying. The Yellow hybrid (No.178) contains higher concentrations from lutein, zeaxanthin, b-cryptoxanthin and b-caotene compared with Yellow hybrid (No.352) and High Tec. White (No.2031). Generally, provitamin A carotenoid constitute 7.65-39.34 % of total carotenoids in maize, whereas zeaxanthin and lutein each commonly represent 30–50 %. Results show that the provitamin A carotenoid compose one third and xanthophyll carotenoids reached to be two thirds. The amounts of provitamin A in traditional yellow maize varieties range from 0.25 µg to 2.5 µg g^{-1} dry weight. Results obtained here, indicate that spraying with polyisoprenic led to the accumulation of xanthophylls with 60% and carotenes with 40%. The precursors of vitamin A are less and this is due to the genotypes behavior under study which directed to the accumulation of both xanthophylls and carotenes, but the xanthophylls were predominant and higher.

Keywords: *Zea mays*, foliar application, β-carotene, lutein, xanthophyll, zeaxanthin.

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1. Introduction

Maize (*Zea mays* L.) is considered the most important crop for the Egyptians after wheat, because it is consumed in the production of bread. Maize ranks as the third most important cereal grain in the world after wheat and rice. Traditional criteria for selecting maize hybrids have been based primarily on agronomic factors, including grain production, disease resistance, drought tolerance and storage characteristics. Little emphasis has been placed on the nutritional value of maize for food and feed. While the majority of the product in developing countries like Egypt is for human consumption, in the developed world it is mainly used for industrial purposes and animal feed (FAO, 1992). Because of its importance, the genetic improvement of maize has played a key role in the development of genotypes with high technological and nutritional values. Specialty maize hybrids are the result of selection for improved chemical composition of the grain compared to standard hybrids. Many of these hybrids including high lysine, high oil, waxy, white and sugary, among others, have been the subject of a renewed interest because of their improvements in agronomic performances, commitments by marketers to preserve the identity of specialty grain, and the advance in our understanding of digestion and nutrient requirements (Mahmoud *et al*., 2020). Despite acting as accessory pigments for photosynthesis, carotenoids have multiple functions in plant metabolism (Taiz and Zeiger, 2010). In particular, they act as potential antioxidants in plants. β-carotene has been reported as a key thylakoid membrane bound antioxidant that can scavenge singlet oxygen species generated from the interaction of P680 and O_2 in photosystem II (Taiz and Zeiger, 2010). Of different carotenoids, β-carotene has been reported to be a bio-stimulant which is thought to be involved in promoting plant growth (Yang *et al*., 2002). Phytate is generally regarded as an important source of nutrients during seed germination, supplying inorganic phosphate (P_i), minerals and myo-inositol to the growing seedling, although it apparently is not required at the levels typical of normal seeds. Furthermore, evidence of accumulating that phytate can also be beneficial to human health, as an antioxidant and anticarcinogen (Zhou and Erdman, 1995). These aspects attest to the importance of phytate, whether or not it is regarded as a desirable compound. Carotenoids are a family of more than 600 liposoluble compounds found in plants, microorganisms, animals and some invertebrates (Britton, 2005). About 50 carotenoids have pro-vitamin A activity, and the β-carotene has the highest activity (Rodriguez-Amaya and Kimura 2004) and for this reason, has been considered as a compound of interest in breeding programs for generation of biofortified crops, especially in edible portions of these plants. These compounds have great importance for human health, acting in mechanisms related to cancer, cardiovascular disease and macular degeneration prevention in humans as well as vitamin A deficiency (FAO, 2002; Rios *et al*., 2011; Vallabhaneni and Wurtzel, 2009). Maize is a carotenogenic plant (Rodriguez-Amaya, 2001) and a staple food for more than 1 billion and 200 million consumers in Africa and Latin America regions where more than 50 millions people present vitamin A deficiency (Cimmyt, 2013). The carotenoids present in corn grain are classified into carotenes (β-carotene and α-carotene) and xanthophylls (lutein, zeaxanthin and β-criptoxanthin), with higher concentrations of lutein and zeaxanthin compared to other carotenoids (Kurilich and Juvik, 1999). Changes in carotenoid profile in the corn kernel have been reported with direct influence of genotype x environment interaction (Kurilich and Juvik, 1999; Rios, 2008) existing relationship between the yellow or orange color of the endosperm and the presence of carotenoids (Chandler *et al*., 2013). However, little information is available in the literature that allows relating the intensity of the grain color and the profile of carotenoids and other fractions of biological relevance to human health. The phenotypic selection based on ear color could provide greater speed and economy in breeding programs aiming at the increase of carotenoids in corn, since the chemical analyses for the quantitation of these compounds are costly and time consuming (Rios *et al.*, 2014). This study was aimed to: (1) evaluating the effect of foliar spraying application during growth of yellow maize, (2) determining the major carotenoids in 3 hybrids of maize, (3) determining the effect of foliar spraying on the approximate composition and carotenoid content of 2 yellow maize and one white hybrid maize.

2. Materials and methods

2.1 Preparation of samples

Four samples of maize grains (*Zea mays* L.) were kindly provided from Prof. Dr. Mohammed El-Mahdy, Sides Station, Agricultural Research Center, Giza, Egypt. Three hybrids of them are colored with yellow or orange husks namely, Yellow hybrid (No.352), Yellow hybrid (No.178), Yellow hybrid (No.176) and High Tec. White (No.2031). The dry grains were purified and sieved, then grind it well, smooth it well, and prepare fine flour for the various extraction process using appropriate solvents.

2.2 Foliar spraying application

Two freshly prepared solutions with final concentrations of 7.5 µg/mL and 15 µg/mL from pure β-carotene powder (Sigma Co.) were dissolved in ethanol (1.0 ml) and transformed to 1L deionized H_2O. The final solutions were filled in compact spraying pumps, and the growing plants sprayed twice, the first one after 45 day from planting and the second one was in the milk stage used as spraying mixture prepared to use them in foliar spraying.

2.3 Preparation of natural carotenoids extracts

An accurate weight, 250 g maize grains from variety yellow hybrid (No.176) colored yellow-husks) was soaked in ethanol for 12 hrs and the yellow extract was obtained by filtration and the solvent let to be evaporated and the stick residue and weighted. Second portion was boiled with methanol under refluxing system then the grains removed and the colored extract cooled and the methanol evaporated and the dried extract was weighted. The third portion was soaked with chloroform for 12 hrs, then the grains removed and the dried extract weighted. Three extracts were resolved in 1 ml chloroform and 0.1 ml from each extract was mixed and transformed to liter deionized-H_2O and the final mixture was used as spraying agent and designated as natural carotenoids extracts (NCE) (Figure 1).

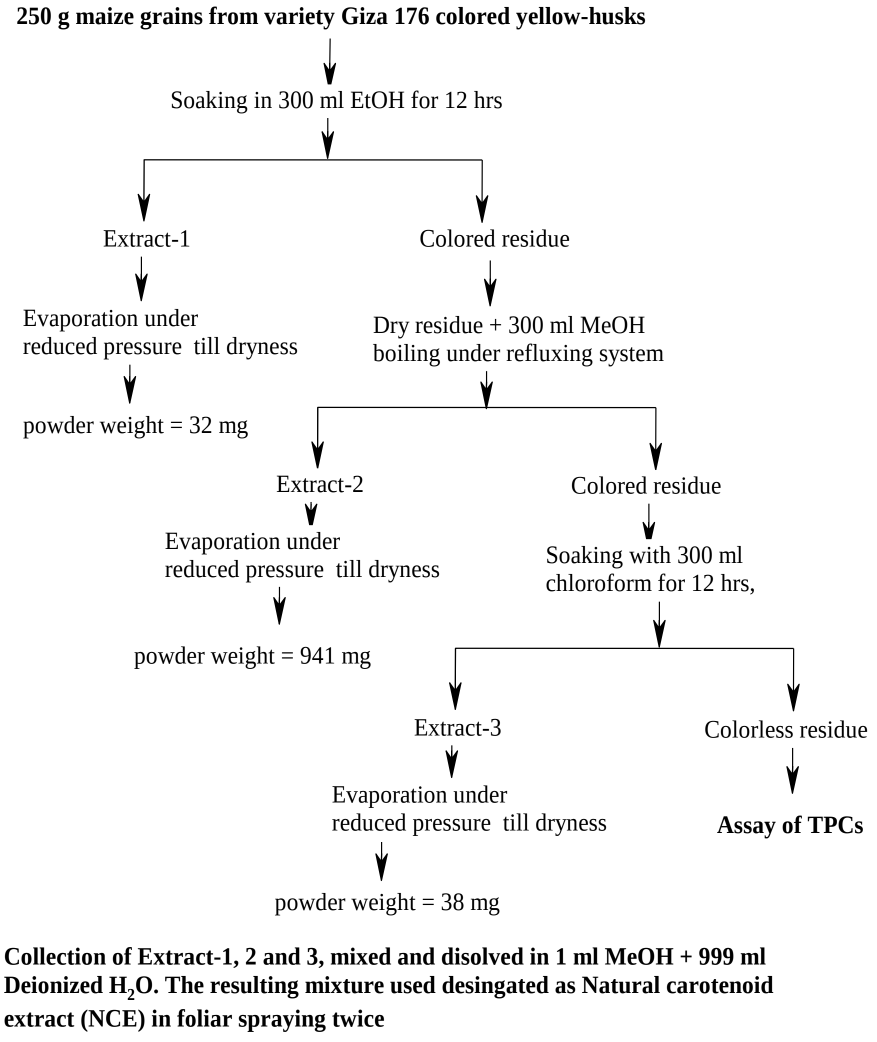

Figure (1): Preparation of natural carotenoids extracts.

Treatments were: Untreated = unsprayed maize plants, 15 ppm β-C= maize plants sprayed 15 µg/mL β-carotene twice after 45 after planting and milk phase, 15 ppm β-C= maize plants sprayed 15 µg/mL β-carotene twice after 45 after planting and milk phase, and carotenoids extract= plants sprayed with natural carotenoid extracts (NCE) twice after 45days after planting and in the milk phase.

2.4 Mineral analysis

The mineral contents of sodium and potassium were determined by flame photometry (Jenway PFP7).

2.5 Chemical composition

Chemical composition of dry maize grains (Moisture content, total ash content, crude protein (% N x 5.75), total crude lipids (TCL) were determined according to AOAC (2000). All determinations were performed in triplicates and the means were calculated.

2.6 Extraction and determination sugars

Extraction and determination of total soluble sugars (TSS) content was determined using the phenol-sulfuric acid method according to Dubois *et al*. (1956). Total reducing sugars (TRS) was extracted and determined according the method described by Miller (1959). The difference between the percentage of total soluble sugars and total reducing sugars was taken as the percentage of non-reducing sugars.

2.7 Extraction and determination of phytic acid

The phytic acid was extracted by the method described by Ellis *et al*. (1977) and determined according to the method of Mohamed *et al*. (1986).

2.8 Extraction and determination of total carotenoids content

Carotenoids content in samples were extracted by first placing one g flour in a glass, adding 50 ml acetone and agitating for 12 h to extract the pigments. Total carotenoid contents were determined according to Saric *et al*. (1967).

2.9 Determination of the carotenoid fractions

The extraction method described by Christian *et al.*, (2000) was followed (Table 1).

Table (1): Determination of carotenoids.

Carotenoid	Calibration curve	R2	Lower detection limit
Lutein	$y = 217049x - 13115$	0.991	12.5 ng
Zeaxanthin	$y = 460837x + 931.98$	0.996	1.2 ng
β-Cryptoxanthin	$y = 148291x - 23.173$	0.997	8.7 ng
β-Carotene	$y = 493623x - 18.374$	0.988	15.1 ng

3. Results and Discussion

3.1 Preparation of natural carotenoids extracts

Results of use of three different solvents namely ethanol, boiling methanol and chloroform to extract of indigenous carotenoids from yellow hybrid (No.176) husks are given in Figure (2). Results indicate that the most efficient solvent is boiling methanol whereas the extract weight reached to be 0.9130 and 0.9692 g/250 g dry weight grains followed by chloroform and ethanol.

3.1.1 Effect of foliar application with β-carotene and natural carotenoids extract on moisture content of three corn hybrids

Moisture content (%) in whole grains of maize hybrids ranged from 9.11 to 10.89% and the highest levels are recorded in untreated samples and the lowest values recorded after the spraying with 7.5 and 15 ppm b-carotenes (βC) (Figure 3). The results also show that the differences in moisture content are close. The result show that foliar spraying increase the dry matter, this effect led to improve the storage ability.

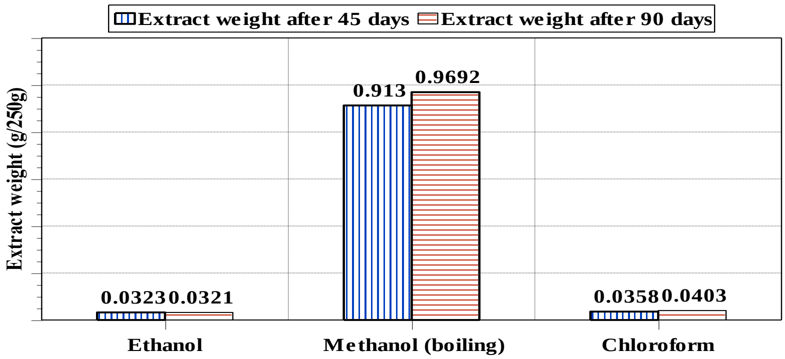

Figure (2): Effect of different solvent ethanol, methanol and chloroform to extract indigenous carotenoids.

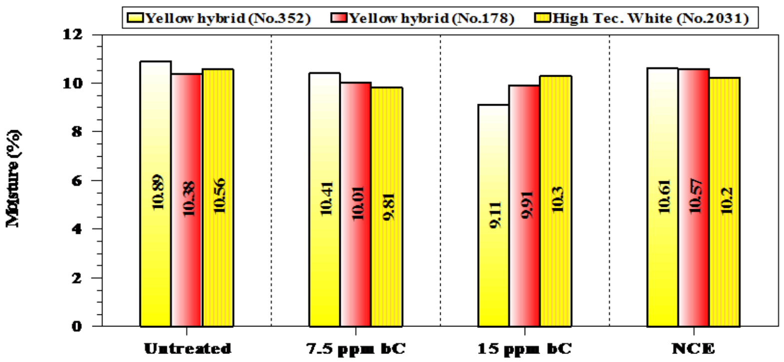

Figure (3): Effect of foliar application with β-carotene and natural carotenoids extract on moisture content of three corn hybrids.

3.1.2 Effect of foliar application with β-carotene and natural carotenoids extract on total ash content (TAC %) of three corn hybrids

Effect of foliar spraying with carotenoids on total ash content was studied and the results indicate that TAC % increased slightly when 15 ppm (βC) was used whereas the use of NCE don't alter the TAC levels in comparison with untreated sample (Figure 4). The yellow hybrid (No.352) and yellow hybrid (No.178) are higher response to spraying with carotenoids than the white one. Obtained results are lower than those reported by Mendoza *et al*. (1998). Results of the spraying effect with carotenoids on sodium and potassium (ppm) of three corn hybrids showed that Yellow hybrid (No.352) contain the highest level of Na and K and the lowest ones in High Tec. White (No.2031) (Table 2). The treatment with 15 μg/mL β-carotene is the most effective treatment to increase metals.

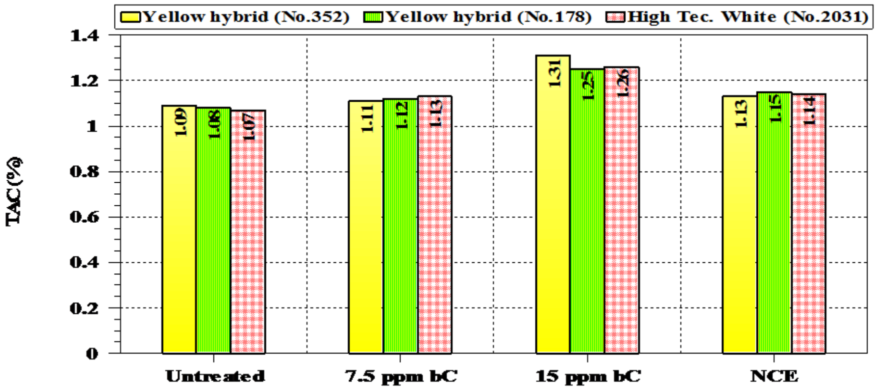

Figure (4): Effect of foliar application with β-carotene and natural carotenoids extract on total ash content (TAC %) of three corn hybrids.

Table (2): Effect of foliar application with β-carotene and natural carotenoids extract on Na^+ and K^+ of three corn hybrids.

Element (ppm)	Concentration	Yellow hybrid (No.352)	Yellow hybrid (No.178)	High Tec. White (No.2031)
Na^+	Untreated	1.4±0.014	0.5±0.004	0.2±0.002
	7.5 μg/mL β-carotene	1.1±0.011	0.3±0.003	0.4±0.004
	15 μg/mL β-carotene	3.1±0.258	0.5±0.004	0.5±0.005
	Carotenoids extract	0.5±0.004	0.6±0.005	0.1±0.000
K^+	Untreated	1.6±0.005	0.2±0.002	0.6±0.006
	7.5 μg/mL β-carotene	1.1±0.011	0.8±0.008	2.3±0.228
	15μg/mL β-carotene	1.7±0.06	0.7±0.007	1.7±0.007
	Carotenoids extract	1.0±0.009	1.1±0.011	0.8±0.000

3.1.3 Effect of foliar application with β-carotene and natural carotenoids extract on phytic acid content (inositol hexaphosphate mg/100g) of three corn hybrids

The concentrations of inositol hexaphosphate (mg/100g) in untreated samples of maize hybrids ranged from 656 to 980 mg/100g and the highest levels are reported in Yellow hybrid (No.352) followed by Yellow hybrid (No.178) and the lowest ones are found in High Tec. White (No.2031) (Table 3). Spraying with 15 µg/mL β-carotene led to accumulate higher concentrations than those reported in all other treatments. These results are in agreement with those reported by Mendoza *et al*. (1998) who found that the reduction in the phytic acid content of maize achieved through genetic engineering yielded minimal changes in the content of other components of the cereal grain.

Table (3): Effect of foliar application with β-carotene and natural carotenoids extract on phytic acid content of three corn hybrids.

Phytic acid content	Concentration	Yellow hybrid (No.352)	Yellow hybrid (No.178)	High Tec. White (No.2031)
Inositol hexaphosphate (mg/100g)	Untreated	980±5	850±5	656±3
	7.5 µg/mL β-carotene	990±6	859±4	667±3
	15 µg/mL β-carotene	1090±12	898±5	695±4
	Carotenoids extract	998±11	876±4	682±3
Ca^{+} mg	Untreated	9.35±0.09	6.67±0.06	8.55±0.09
	7.5 µg/mL β-carotene	10.3±0.11	6.74±0.07	8.95±0.09
	15 µg/mL β-carotene	12.5±0.13	9.56±0.11	1125±0.11
	Carotenoids extract	10.4±0.11	6.84±0.09	9.25±0.10
P mg	Untreated	62.1±2.2	64.2±2.2	56.4±1.8
	7.5 µg/mL β-carotene	64.3±2.2	59.9±2.0	53.5±1.7
	15 µg/mL β-carotene	61.2±2.1	57.8±1.9	63.1±2.2
	Carotenoids extract	59.9±2.0	61.2±2.1	59.7±2.0

Generally it is notice the hybrids having high levels of phytic acid are favourable for human nutrition for many reasons. Phytic acid has hypo-cholesterolemic, antioxidative, anticarcinogenic, and hypolipidemic effects and has been suggested to have a role in the prevention of caries and platelet aggregation in the treatment of hypercalciuria and kidney stones (Potter, 1995; Thompson, 1994). Phytic acid also has physiological effects similar to those of high-fiber diets and such as may be partly responsible for some of the health benefits. The function of phytic acid is not only for energy storage and antioxidation of fats of seeds but also for protecting seed from fungal invasion (Dayi *et al*., 1995). In opposite manner, many other scientists (Coelho *et al*., 2002) have reported that phytic acid is the major storage form of phosphorus in seeds of legumes and cereals. Since phytate can form complexes with proteins and minerals thereby reducing the digestive availability of these nutrients, It is usually regarded as an antinutrient, although recent work indicates that it has important beneficial roles as an antioxidant and anticarcinogen. Therefore, there is an interest in the assessment and manipulation of phytate contents in important food grains such as beans. In recent years, there has also been concern that phytate may be a major contributor to phosphate pollution (Raboy, 2003).

3.1.4 Effect of foliar application with β-carotene and natural carotenoids extract on total crude protein (TCP) (mg/g) of three corn hybrids

Crude protein in the whole maize grains ranged from 19.7 to 36.7 mg/g and the highest levels recorded in yellow hybrid (No. 352) sample and the lowest one in untreated one (Figure 5). Spraying with 15 ppm β-carotene led to the highest values of crude protein. These results are not in agreement with those reported by Mendoza *et al*. (1998) who found that protein content in processed and unprocessed maize grains ranged from 8.1 to 9.0 g.

3.1.5 Effect of foliar application with β-carotene and natural carotenoids on total crude lipids of three maize hybrids

Results of the application of β- carotene and NCE on crude lipids show that yellow hybrid (No. 352) recorded the highest levels followed by yellow hybrid (No. 178) and the lowest levels are recorded in high tec. White (No. 2130). The concentrations with 7.5 and 15 β-carotene ppm are better than NCE (Figure 6).

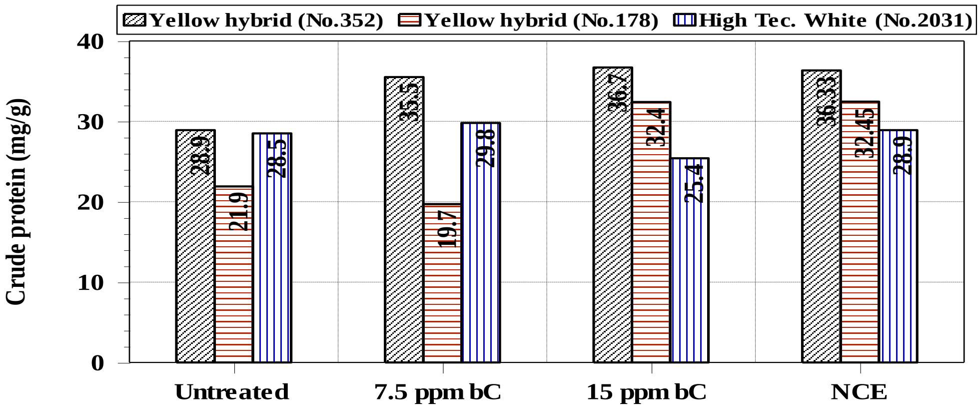

Figure (5): Effect of foliar application with β-carotene and natural carotenoids extract on crude protein (mg/g) of three corn hybrids.

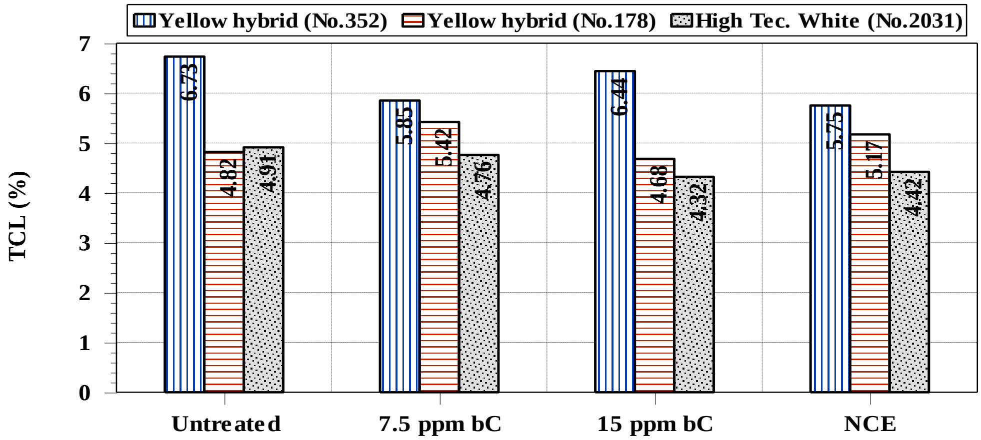

Figure (6): Effect of foliar application with β-carotene and natural carotenoids extract on total crude lipids of three corn hybrids.

It could be concluded that response of lipids in maize hybrids for foliar application are low. These results are in a good agreement with reported by Mendoza *et al*. (1998) who found that the ether extract of processed and unprocessed maize samples ranged from 4.0 to 4.8 g.

3.1.6 Effect of foliar application with β-carotene and natural carotenoids on total soluble sugars (TSS), total reducing sugars (TRS) and total non-reducing sugars (TNRS) of three corn hybrids

Results in Table (4) indicate that the sugars contents are in close extents. Total reducing sugars (TRS) in all hybrids and treatments are more predominate than non-reducing ones. The colored maize hybrids (No. 352) and hybrids (No. 178) contain higher levels from total soluble sugars and total reducing sugars than those reported in High Tec. White (No. 2130) (Figure 7). Spraying with 15 ppm b-carotene led to the highest level of total soluble sugars.

3.1.7 Effect of foliar application with β-carotene and natural carotenoids on lutein, zeaxanthin, β-cryptoxanthin and β-carotene and total carotenoids (Cs) of three corn hybrids

The yellow corn genotypes studied here are characterized with high levels of different isoforms of carotenes and xanthophylls and the results existenced in Table (4) and Figure (8). The tabulated data showed that the most predominant carotene is b-carotene and the three predominant xanthophyll are zeaxanthin followed by lutein and b-cryptoxanthin. Total carotenoids in untreated and treated samples ranged from 47.63 to 568.49 mg/g and the highest concentration was recorded when 15 ppm b-carotene was used as a foliar spraying. The Yellow hybrid (No. 178) contains higher concentrations from lutein, zeaxanthin, b-cryptoxanthin and b-caotene when compared with Yellow hybrid (No. 352) and High Tec. White (No. 2031). Generally, provitamin A carotenoid constitute 7.65–39.34 % of total carotenoids in maize, whereas zeaxanthin and lutein each commonly represent 30–50 %.

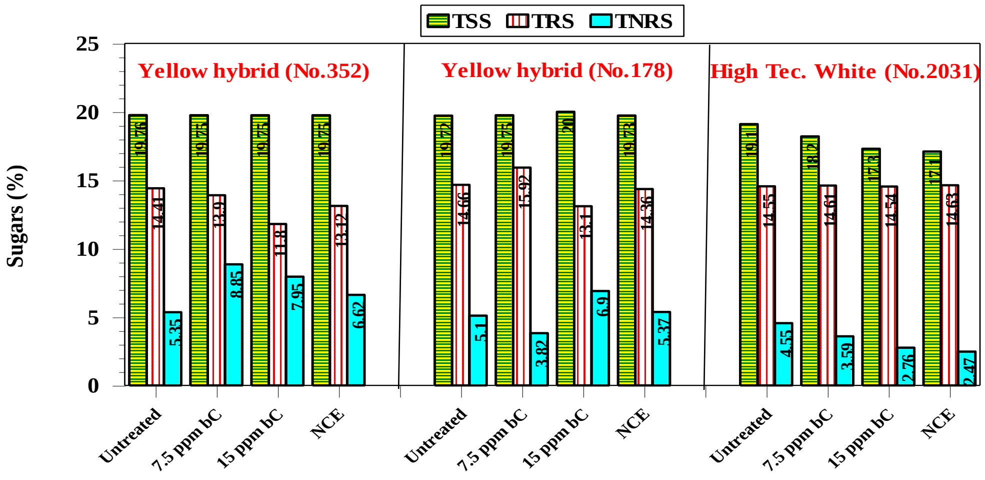

Figure (7): Effect of foliar application with β–carotene and natural carotenoids extract on TSS, TRS and TNRS of three corn hybrids.

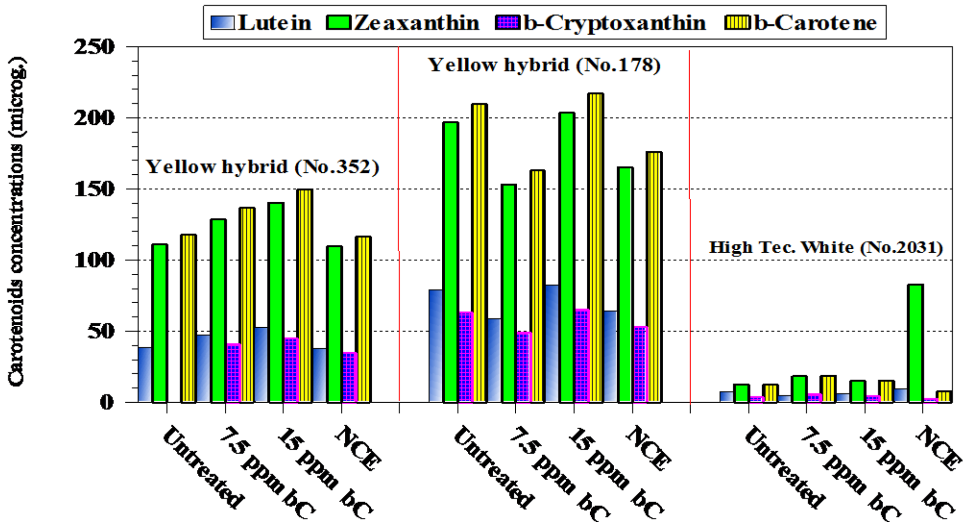

Figure (8): Effect of foliar application with β-carotene and natural carotenoids on concentrations among maize hybrids.

Table (4): Effect of foliar application on chemical composition of dry *Zea mays* hybrids.

Constitutes (%)	Concentration	Yellow hybrid (No.352)	Yellow hybrid (No.178)	High Tec. White (No.2031)
Moisture (%)	Untreated	10.89±0.18	10.38±0.14	10.56±0.16
	7.5 µg/mL β-carotene	10.40±0.15	10.01±0.11	9.80±0.11
	15 µg/mL β-carotene	9.00±0.09	9.90±0.11	10.30±0.15
	Carotenoids extract	10.60±0.16	10.57±0.16	10.20±0.14
TAC	Untreated	1.09±0.01	1.08±0.01	1.07±0.01
	7.5 µg/mL β-carotene	1.11±0.02	1.12±0.02	1.13±0.02
	15 µg/mL β-carotene	1.20±0.02	1.09±0.02	1.08±0.02
	Carotenoids extract	1.13±0.04	1.15±0.04	1.14±0.04
TCL	Untreated	6.73±0.31	4.82±0.25	4.90±0.27
	7.5 µg/mL β-carotene	5.85±0.28	5.42±0.26	4.76±0.22
	15 µg/mL β-carotene	6.44±0.30	4.68±0.24	4.32±0.22
	Carotenoids extract	5.75±0.27	5.17±0.25	4.42±0.21
TCP (mg/g)	Untreated	28.90±1.4	21.90±1.3	28.50±1.3
	7.5 µg/mL β-carotene	35.50±2.5	19.70±1.2	29.80±1.4
	15 µg/mL β-carotene	36.70±2.6	32.40±2.4	25.40±1.5
	Carotenoids extract	36.33±2.6	32.45±2.2	28.90±1.5
TSS	Untreated	19.76±1.2	19.72±1.2	19.70±1.2
	7.5 µg/mL β-carotene	19.75±1.2	19.75±1.2	19.75±1.2
	15 µg/mL β-carotene	19.75±1.2	19.73±1.2	19.71±1.2
	Carotenoids extract	19.75±1.2	19.73±1.2	19.76±1.2
TRS	Untreated	14.41±1.1	14.66±1.1	14.52±1.1
	7.5 µg/mL β-carotene	13.90±1.1	15.92±1.1	14.61±1.1
	15 µg/mL β-carotene	11.80±1.1	12.93±1.1	14.54±1.2
	Carotenoids extract	13.12±1.2	14.36±1.2	14.63±1.2
TNRS calculated	Untreated	5.35	5.06	5.18
	7.5 µg/mL β-carotene	5.85	3.83	5.14
	15 µg/mL β-carotene	7.95	6.80	5.17
	Carotenoids extract	6.63	5.37	5.13

Table (5): Effect of foliar application with β-carotene and natural carotenoids on concentrations among treated maize hybrids.

Hybrids	Treatments	Carotenoid concentrations (µg)				
		Lutein	Zeaxanthin	β-cryptoxanthin	β-carotene	Total Cs
Yellow hybrid (No.352)	Untreated	38.76	111.10	35.42.	117.96	303.24
	7.5 ppm βC	47.10	128.6	41.05	136.72	353.47
	15 ppm βC	52.65	140.6	44.91	149.55	387.71
	NCE	38.19	109.7	34.97	116.48	299.34
Yellow hybrid (No.178)	Untreated	79.14	196.80	63.00	209.77	548.71
	7.5 ppm βC	58.51	153.10	48.91	162.88	423.4
	15 ppm βC	82.39	203.70	65.22	217.18	568.49
	NCE	64.37	165.50	52.92	176.21	459.00
High Tec. White (No.2031)	Untreated	7.69	12.50	3.68	12.33	36.20
	7.5 ppm βC	4.87	18.40	5.62	18.74	47.63
	15 ppm βC	6.39	15.20	4.58	15.28	41.45
	NCE	9.64	83.10	2.35	7.88	102.97

3.2 The provitamin A carotenoid

Results presented in Table (6) and Figure (8) show that the provitamin A carotenoid compose one third and xanthophyll carotenoids reached to be two thirds. The amounts of provitamin A in traditional yellow maize varieties range from 0.25 µg to 2.5 µg /g dry weight. These results are disagreed with those reported by Watson (1962) and Weber (1987). Results of Muzhingi *et al*. (2008) indicated that carotenoid extraction without saponification showed a significantly higher yield than that obtained using saponification. They added that yellow maize is a good source of provitamin A carotenoids and xanthophylls. Cooking by boiling yellow maize at 100 degrees C for 30 minutes increased the carotenoid concentration, while baking at 450 degrees F for 25 minutes decreased the carotenoid concentrations by almost 70% as compared to the uncooked yellow maize flour. In a work carried out by Lokaewmanee *et al.* (2010) to study the effects on egg yolk color of paprika or paprika combined with marigold flower extracts. They reported that total carotenoid and total xanthophyll in yellow corn were 0.03 mg/g and 0.04 mg/g respectively while lutein, zeaxanthin and cryptoxanthin contents were 0.005, 0.010 and 0.003 mg/g respectively.

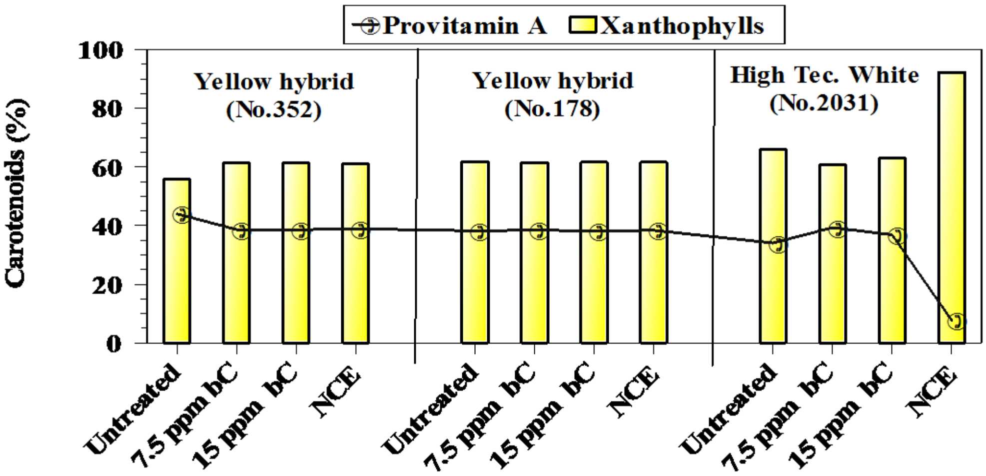

Figure (7): Effect of foliar application with β-carotene and natural carotenoids extract on provitamin A and common xanthophylls of three corn hybrids.

Table (6): Effect of foliar application with β-carotene and natural carotenoids extract on provitamin A and common xanthophylls of three corn hybrids.

Treatment	β-Carotene / Total Cs (Provitamin A (%)	Lutein+zeaxanthin+β-cryptoxanthin xanthophylls/ Total (Cs) X 100 (%)
Untreated	38.90	61.10
7.5 ppm βC	38.68	61.32
15 ppm βC	38.57	61.43
NCE	38.91	61.09
Untreated	38.23	61.77
7.5 ppm βC	38.47	61.80
15 ppm βC	38.20	61.78
NCE	38.39	61.61
Untreated	34.06	65.94
7.5 ppm βC	39.34	60.66
15 ppm βC	36.86	63.14
NCE	7.65	92.35

These results are lower than our results reported in the current work. Usually, yellow flint maize has a high content of proteins and b-carotene. In other maize types b -carotene with the highest pro-vitamin A activity is present in a relatively low concentration (Cortés *et al.,* 2006; Mahmoud *et al*., 2020). Yellow maize kernel carotenoids are present in different isoforms, including two carotenes, α- and β-carotene, and three xanthophylls, β-cryptoxanthin, zeaxanthin, and lutein (Watson, 1962) and (Weber, 1987). The predominant carotenoids in maize kernels, in decreasing order of concentration, are lutein, zeaxanthin, β-carotene, β-cryptoxanthin, and α-carotene. Generally, provitamin A carotenoids constitute only 10–20 % of total carotenoids in maize, whereas zeaxanthin and lutein each commonly represent 30–50 %. The amounts of provitamin A in traditional yellow maize varieties range from 0.25 μg to 2.5 μg g _1 dry weight. Maize exhibits considerable natural variation for kernel carotenoids, with some genotypes accumulating as high as 66.0 μg/g (Harjes *et al*., 2008). In typical maize, concentrations of provitamin A carotenoids, i.e., α-carotene, β-carotene, and β-cryptoxanthin, range from 0 to 1.3, 0.13 to 2.7, and 0.13 to 1.9 nmol/g, respectively Kurilich and Juvik (1999). Although β-carotene has the highest provitamin A activity, it is present in a relatively low concentration (0.5–1.5 μg/g) in most yellow maize grown and consumed throughout the world (Harjes *et al.,* 2008). Results reported by Berardoa *et al.,* (2004) provided evidence that there were wide differences among kernel samples for all carotenoid components examined: violaxanthin, lutein, zeaxanthin, isolutein, cryptoxanthin and carotenes. Lutein and zeaxanthin, the prevalent carotenoids present in the maize kernels, ranged, respectively, from not detectable (Quarantino bianco) to 29.70 (Lo1010-Lo1058) and from 0.5 (Quarantino bianco) to 38.20 mg kg^{-1} (Quarantino giallo). Lutein and zeaxanthin, the dominant carotenoids in maize kernels, are the essential components of the macular pigment of the eye (Beatty *et al*., 1999; Mahmoud *et al*., 2020).

4. Conclusion

It could be concluded that the recorded values for some parameters are highest in genotypes 178 and spraying with 15 µg/mL of β-carotene resulted in increases in the concentration of lutein, zeaxanthin, β-cryptoxanthin, and β-carotene. From the results obtained, it is clear that spraying with β-carotene led to the accumulation of xanthophylls (60%) and β-carotene (40%), this means the pro-vitamin A is less and this is due to the genotypes behavior under study directed to the accumulation of both xanthophylls and β-carotene, but the xanthophylls were higher and predominant. We recommend spraying application with 15 µg/ml twice the first one after 40 days of transplantation and the other one during the milk phase, to increase the concentration of zeaxanthin, lutein, β-cryptoxanthin and β-carotene. This field needs further studies in the future.

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