Egypt. J. Plant Breed. 20(5):705 – 722 (2016)

IMPROVEMENT OF SWEET CORN BIOFORTIFICATION FOR β-CAROTENE CONTENT BY USING FIELD CORN IN EGYPT

H.E.M. Zaki

Hort. Dept, Fac. of Agri., Minia University, El-Minia, Egypt

ABSTRACT

Highly significant differences were observed for the kernels and flour-Hunter b*values in the evaluated genotypes of 7 sweet corn, 8 field corn and 3 F_1 field-sweet corn crosses. Flour yellowness index of F_1 plants was between the mid-parent in the cross between inbred 2605-1288Y (yellow field corn "P_{16}") and Country Gentleman cv. (white sweet corn "P_{19}") and lower parent in the cross between inbred 82 (white field corn "P_{12}") and (P_{19}) but higher than the better-parent in cross between (P_{12}) and Golden Bantam cv. (yellow sweet corn "P_{20}"). HPLC results showed that F_1 of field-sweet corn crosses significantly varied in α and β-carotene. F_1 of (P_{12}xP_{20}) had the highest concentrations of zeaxanthin and β-cryptoxanthin when F_1 of (P_{16}xP_{19}) and (P_{12}xP_9) accumulated much more α and β-carotene. The inheritance of yellowness index was studied in (P_{16}xP_{19}) and (P_{12}xP_{20}) crosses using the data of parents, F_1, F_2 and backcrosses populations. Non- and additive gene effects were significantly contributed to yellowness index with greater governing effects to the non-additive gene. A partial dominance towards the lower parent was involved in the inheritance of this trait and heritability estimates were relatively high in broad and narrow sense. However, there was non-significant correlation between the grain color and β-carotene, polymorphisms of lcyE among genotypes had significant differences either for α- or β-branch carotenoids. The two F_1 field-sweet corn crosses; (P_{12}xP_9) and (P_{16}xP_{19}) which showed high β-carotene content got amplification of lcyE. The obtained results are a step to produce more adapted and healthier sweet corn in Egypt.

Keywords: ***Zea mays var. rugosa, Field-sweet corn crosses, β-carotene content, Lycopene epsilon cyclase (lcyE).***

INTRODUCTION

Vitamin "A" deficiency is a major world health problem, affecting more than 127 million pre-school children and 7 million pregnant women worldwide (West 2003). However, this can be alleviated through provitamin "A" carotenoid biofortification of major crop staples such as maize. Maize (*Zea mays* L) or corn has long been one of the most important food crops in the world being grown for both human and animal consumption (Farnham *et al* 2003). As a global staple crop with considerable variation in kernel carotenoid composition, maize could have widespread impact (Owens *et al* 2014). Sweet corn, a form of maize, is an important and popular vegetable, especially in the United States where about 250,000 ha are grown annually and North America dominates world sweet corn production. The popularity of sweet corn has greatly increased and there is an apparent trend for further expansion of production in European and Asian countries, especially Japan and China (Rubatzky and Yamaguchi 1997). Because of the importance of this crop, corn breeders have focused a great deal of attention on improvement of a number of generally valuable characteristics, such as

yield, tolerance of environmental stress, disease and insect resistance (Tracy 1997) and carotenoids content through biofortification (Harjes *et al* 2008).

Biofortification, which means breeding plants with increasing their nutritional values, is one of the most important strategies to avoid or decrease nutrient deficiencies (Welch and Graham 2002 and Bouis *et al* 2011). Many protocols have been initiated to enrich the level of nutritional values in many crops. The Harvest Plus Program of the Consultative Group on International Agricultural Research (CGIAR), for example, works to reduce the effects of micronutrient malnutrition by harnessing the power of plant breeding to develop staple foods such as rice, sweet potatoes, maize, cassava, wheat and beans that are rich in iron, zinc or provitamin "A" (HarvestPlus 2004 and Bouis *et al* 2011).

Carotenoids are essential to many aspects of human and animal health, yet they do not synthesize carotenoids (Moran and Jarvik 2010), and therefore must obtain them from their diet to meet minimal nutritional requirements. The most important provitamin "A" carotenoids in plant-based foods are β-carotene (two retinyl groups), β-cryptoxanthin (one retinyl group), and α-carotene (one retinyl group), but in most plant tissues they are substrates for hydroxylation reactions that produce the dihydroxyxanthophylls; lutein and zeaxanthin (Fig. 1), the most prevalent carotenoids in vegetative and seed tissues (Howitt and Pogson 2006 and Cazzonelli and Pogson 2010).

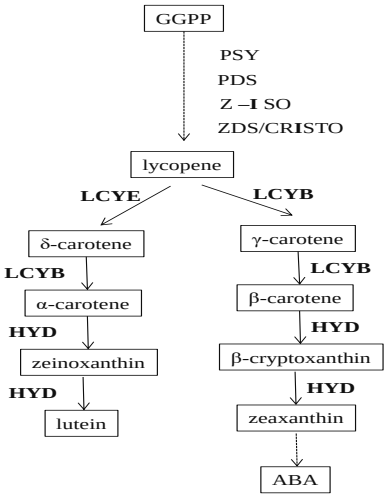

Fig. 1. Carotenoid biosynthetic pathway in plants (Matthews and Wurtzel 2007). Reactions are represented by arrows, dashed arrows represent multiple enzymatic steps. Compounds: GGPP, geranylgeranyl diphosphate; ABA, abscisic acid. Enzymes: PSY, phytoene synthase; PDS, phytoene desaturase; Z-ISO, 15-*cis* zetacarotene isomerase; ZDS, zetacarotene desaturase; CRTISO, carotene isomerase; HYD, carotene hydroxylase enzymes, which include ε- and β-ring hydroxylases.

Carotenoids are derived from the isoprenoid biosynthetic pathway and are precursors of the plant hormone abscisic acid and of other apocarotenoids (Harjes *et al* 2008). The first committed step of this pathway is formation of phytoene from geranylgeranyl diphosphate by phytoene synthase (*y1/psy1*). The first branch point of this pathway occurs at cyclization of lycopene where action of lycopene beta cyclase (*lcyB*) at both ends of linear lycopene produces a molecule with two β rings. Alternatively, the coaction of *lcyB* and lycopene epsilon cyclase (*lcyE*) generates α, β and ε- carotene that is a precursor to lutein. Relative activities of *lcyB* and *lcyE* are hypothesized to regulate the proportion of carotenes directed to each branch of this pathway. Indeed, transgenic manipulations of *lcyE* expression in *Arabidopsis*, potato, brassica and maize increase the pool of β ring–containing carotenes (Fig. 1).

In general, maize displays considerable natural variation for carotenoid composition, including vitamin "A" precursors; α-carotene, β-carotene, and β-cryptoxanthin. One of the biggest challenges of breeding proA biofortified maize is the low throughput and high cost of quantifying carotenoid content in maize grain (Bouis 2003). Field corn is more adapted with the Egyptian environment however; sweet corn is accounted as a new crop and grown in small areas (Wahba *et al* 2015). Therefore, the main objective of this investigation is to use the field corn genes to generally improve the sweet corn especially, β-carotene contents. This was done through the following experiments: (1) measure Hunter b*values for immature kernels and corn flour in 7 sweet corn, 8 field corn genotypes and 3 F_1 field-sweet corn crosses, (2) estimate some genetic parameters of flour yellowness index trait, (3) use quick method of high performance liquid chromatography (HPLC) to screen these genotypes of levels of pro-vitamin "A" and (4) evaluate the phenotypic effects of different functional polymorphisms within *lcyE* in all genotypes.

MATERIALS AND METHODS

Molecular study was conducted at the Laboratory of Plant Breeding, Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan during the period of 26 November 2013 to 25 May 2014. Carotenoids analysis was done at USDA-Agricultural Research Service, Genetic Improvement of Fruits and Vegetables Laboratory, Beltsville, Maryland, USA during the period of 15 October 2012 to 14 April 2013.

Plant materials

Two sweet corn cultivars; Country Gentleman cv. and Golden Bantam cv. (Bountiful Gardens, New jersey, USA) and two field corn inbreds; white field corn inbred 82 and yellow field corn inbred 2605-1288Y (EGAS, Egypt) were used. Also, six yellow field corn inbreds (*Zea mays* var. *indurata*) and five sweet corn inbreds (*Zea mays* L. var. *rugosa* Bonaf) were produced and evaluated in this study. Field corn inbreds and

sweet corn inbreds/cultivars were used as parents of field-sweet hybrids. Six Egyptian field corn inbreds were developed from selfing open-pollinated plants of yellow Yemen's landraces collected by S. H. Gadelhak for five successive generations, and maintained at the Horticulture Department Research Farm, Faculty of Agriculture, Minia University, El-Minia, Egypt. The five sweet corn inbreds were developed by Wahba (2009) and maintained by sibbing mating system. Each inbred was represented by ten plants, which were used for intercrossing and selfing. These materials gave a total of eleven parental inbreds. Producing self and F_1 hybrid seeds were done by bagging hand pollination in 2011 season. Harvesting was done at the full mature stage and the grain-moisture was adjusted at 15.5% and grains were stored in refrigerator at 5 $^{o}C\pm1$.

Kernel color estimations

At the maturity growth stage, the kernel flour color was detected using 25, 100 and 76 random plants from the parents, F_1 and their F_2 and backcrosses populations (BC_1P_1 and BC_1P_2), respectively. To measure Hunter b* readings, the kernels from each plant were collected and air dried. Dry grain samples were powdered using electrical mill for obtaining whole corn flour. Each grain sample was evaluated objectively by scoring its flour Hunter b* parameter using a colorimeter instrument "Color Tec PCM Color Meter Tec. NJ., USA" reflectance spectra model.

HPLC analysis of carotenoids content

Extraction and analysis were modified from Hulshof *et al* (2007). The method described allows fast screening of maize kernels on levels of pro-vitamin "A" without the need of a full HPLC analysis of all samples, and hence reduces the cost of analyses. Ground maize (2g) was soaked overnight in 5ml water at ($4^{o}C$) and then extracted twice with 15ml cold ($4^{o}C$) acetone and once with 25 ml cold ($4^{o}C$) acetone/hexane (60/40 v/v) containing 1 mg/l retinylacetate as an internal standard, using a rod mixer (Polytron PT 20 OD, Kriens/Luzern, Switzerland). The mixture, including maize residue was transferred to a 250ml volumetric flask using water. Additional cold water was added till the full hexane phase (containing the carotenoids) had risen to the small part of the flask. Drain water solution, allow hexan through anhydrous Na_2SO_4. The hexane fraction was transferred into a 10ml Kimax glass tube with PTFE seal screw cap and centrifuged for 5 min at 1580g. An aliquot of 1.0 ml of the clear hexane extract was evaporated under a stream of nitrogen and the residue was dissolved in 1.0ml methanol/tetrahydrofuran (3:1 v/v) containing 0.01% w/v BHT. An amount of 25ml was injected into the HPLC system. A YMC 4.6X250mm 3µm C30 column was used with flow rate of 0.8ml/min run on gradient of methyl-tert-butyl ether (A), methanol (B) and water (C) with 0.005M ammonium acetate and 0.05% triethylamine. Through whole run, C is kept at 1% while the solution A at time 0;5;8;16;18;22;23;26;27;35

minute is keep at 5;15;15;39;45;45;99;99;5;5. All carotenoid standard is from ChromaDex Co., Irving, Calif. USA. The Optima grade methanol, HPLC grade methyl-tert-butyl ether, ammonium acetate and triethylamine are from Fisher scientific, Pittsburg, PA. USA. All sample preparations and extractions were done in duplicate and under subdued light.

DNA isolation and PCR amplification of *lcyE*

Total DNA was extracted from maize seed powder of the plant materials using GenElute TM Plant Genomic DNA Miniprep kit (Sigma, Saint Louis, USA) according to the manufacturer's instructions. Natural genetic variation in (*lcyE*) was analyzed in the inbreds with single nucleotide polymorphism (SNP) and gene specific markers that were reported earlier by Harjes *et al* (2008).

To amplify the loci, the PCR was performed in a 20µl containing 2ng of genomic DNA, 1µl of each primer (10mM), 2µl of dNTPs (10mM), 1µl of 10x reaction buffer (Takara, Japan), and 1 unit of *Taq* polymerase (Takara). The PCR program was set up as: Initial denaturation 94°C for 2 min; 40 cycles of denaturation 94°C for 1 min, annealing Tm°C for 1 min, and extension 72°C for 1 min; and finally 72°C for 4 min. The PCR products were fractionated in a 4% NuSieve 3:1 Agarose (Lonza, Rockland, ME, USA) by using gel electrophoresis apparatus (EIDD, Japan). After electrophoresis, the gel was stained with ethidium bromide and viewed with a UV illuminator. Markers used and annealing temperature are shown on Table 1.

Table 1. Lycopene epsilon cyclase (*lcyE*) specific marker used to detect genetic variation in field corn, sweet corn genotypes and field-sweet corn crosses.

Marker	Tm ºC	Orientation	Sequence (5` to 3`)	Mers	Size (bp)
***lcyE* SNP216**	**60.6**	**FW**	**GCGGCAGTGGGCGTGGAT**	**18**	**395**
		RV	**TGAAGTACGGCTGCAGGACAACG**	**23**	
***lcyE* 5' indels/TE**	**55.0**	**FW**	**CGCTAGCAAGCCCATTATTTTTA**	**23**	**993**
		RV	**CGGTATGGTTTTTGGTATACGG**	**22**	
***lcyE* 3' indel**	**59.9**	**FW**	**GGACCGGAACAGCCAACTG**	**19**	**144**
		RV	**GGCGAAATGGGTACGGCC**	**18**	

Tm ºC= annealing temperature

Statistical analysis

The recorded data were subjected to various statistical methods to shed light upon the dominance, the component of generation means, gene action, heritability and minimum number of operating genes (Azizi *et al* 2006). Also, the scaling test for A, B and C scales was performed according to Mather (1949). Joint scaling test of Cavalli (1952) was applied to estimate mid-parental value (m), dominance (h) and additive (d) gene effects following the method proposed by Mather and Jinks (1971).

Hayman's equations (1958) were used to estimate the six-parameter model to explain the observed variation as suggested by Mather and Jinks (1977):
m= average effect = F_2.
d= additive effect = BC_1P_1- BC_1P_2.
h= dominance effect.
i= additive × additive interaction effect.
j= additive × dominance interaction effect.
l= dominance × dominance interaction effect.

These parameters were tested for significance using t-test with the tabular t values at n-1 degrees of freedom, where n is the number of plants used in estimating the variances of all generation involved. Phenotypic variances of the six generations were used to estimate the heritabilites based on the procedure proposed by Mather and Jinks (1971). Heritability was characterized as low (<30%), moderate (31-60 %) and high (>61%) as described by Robinson *et al* (1949).

All obtained data was statistically analyzed according to Gomez and Gomez (1984) using MSTATC program. The Duncan`s test at 5% level of probability was utilized for the comparisons among the tested genotypes in a randomized complete block design with three replications.

RESULTS AND DISCUTIONS

Results

Hunter b* of immature kernels and corn flour

Means of Hunter b*values for immature kernels and corn flour in genotypes of 7 sweet corn, 8 field corn and 3 F_1 field-sweet corn crosses are shown in Table 2. Highly significant differences were observed among the tested genotypes for the Kernels-Hunter b*value, flour-Hunter b*value, and flour yellowness index. Kernels-Hunter b*value ranged from (18.23 to 41.22), flour-Hunter b*value ranged from (11.11 to32) while, flour yellowness index ranged from (20.21 to 75.57). Inbred 3, a field corn inbred with yellow mature ear exhibited the highest Kernels-Hunter b*value. On the other hand, inbred 6 had the highest and significant flour-Hunter b*value.

For flour yellowness index, yellow genotypes had higher index than white genotypes, regardless the corn type. In the studied crosses, the two parents of each cross differed significantly for Hunter b* readings. F_1 values were between the mid-parent in ($P_{16}xP_{19}$) cross and lower parent in ($P_{12}xP_9$) cross, indicating the recessiveness, however, F_1 values were higher than the better-parent in ($P_{12}xP_{20}$) cross (Table 2).

Table 2. Means of Hunter b*values for immature kernels and corn flour as well as carotenoids content in 7 sweet corn, 8 field corn genotypes and 3 F1 field-sweet corn crosses.

Genotypes	Type[1]	Mature ear color	Kernels-Hunter b*value	Flour-Hunter b* value	Flour yellowness index[2]	Carotenoids contents (µg/g)			
						Zeaxanthin	β-cryptoxanthin	α-carotene	β-carotene
Inbred 1	F	Yellow	39.1 ab	27.10 de	52.62 ab	3.672 g	0.456 hi	1.625 c	1.125 d
Inbred 2	F	Yellow	32.2 ef	25.48 ef	45.82 ab	2.541 i	0.377 i	2.218 b	1.801 c
Inbred 3	F	Yellow	41.22 a	26.73 e	50.00 ab	3.380 gh	0.675 gh	0.700 efg	0.149 e
Inbred 4	F	Yellow	33.97 de	30.99 ab	75.57 ab	4.153 g	0.878 g	0.887 e	0.270 e
Inbred 5	F	Yellow	34.56 de	29.92 bc	56.66 a	2.876 hi	0.756 g	0.813 ef	0.222 e
Inbred 6	F	Yellow	32.28 ef	32.03 a	60.10 a	3.642 g	0.872 g	2.145 b	1.741 c
Inbred 7	S	White	30.03 fg	24.51 fg	45.96 ab	1.484 j	0.110 j	0.458 gh	0.112 e
Inbred 8	S	White	31.34 f	24.08 fgh	45.50 ab	1.654 j	0.688 gh	0.347 h	0.147 e
Inbred 9	S	White	36.1 cd	22.43 h	43.02 abc	17.178 a	0.798 g	0.727 ef	0.126 e
Inbred 10	S	White	27.38 h	25.77 ef	49.13 ab	1.278 j	0.136 j	0.446 gh	0.177 e
Inbred 11	S	White	21.63 i	23.41 gh	43.13 abc	5.241 f	0.130 f	0.562 fgh	0.257 e
Inbred 82 (P_{12})	F	White	18.79 j	11.11 k	20.21 d	9.202 d	2.021 de	1.291 d	0.207 e
Inbred 2605-1288y (P_{16})	F	Yellow	31.06 f	25.62 ef	48.93 ab	7.656 e	1.839 e	1.772 c	1.692 c
Country Gentleman (P_{19})	S	White	28.07 gh	13.81 j	27.59 cd	11.615 c	2.478 b	0.332 h	0.164 e
Golden Bantam (P_{20})	S	Yellow	27.32 h	25.52 ef	54.05 a	12.582 b	2.297 bc	0.947 e	0.266 e
F_1 (P_{12}xP_9)	FxS	White	18.23 j	12.46 jk	28.57 cd	7.992 e	2.151 cd	3.799 a	3.265 a
F_1 (P_{12}xP_{20})	FxS	Yellow	37.95 bc	28.23 cd	46.36 ab	11.492 c	2.925 a	1.810 c	0.286 e
F_1 (P_{16}xP_{19})	FxS	Yellow	27.45 h	18.17 j	36.30 bc	9.456 d	2.769 a	2.329 b	2.512 b

Means in the same column followed by similar letter or letters are not significant at 0.05 level by Duncan's New Multiple Range Test.

[1] Type S= Sweet corn F= Field corn FxS= Field corn x Sweet corn

[2] YI = (142.86*Hunter-b*value)/Hunter L value

HPLC analysis of carotenoids content

The concentration of zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene using HPLC revealed the existence of considerable variation among genotypes in the carotenoids contents. Table 2 and Fig. 2 show the carotenoid content and profile in the evaluated field and sweet corn genotypes. Zeaxanthin and β-Cryptoxanthin concentrations ranged from 1.278µg/g in inbred 10 (white sweet corn inbred) to 17.178 µg/g in inbred 9 (white sweet corn inbred) and from 0.11 µg/g in inbred 7 (white sweet corn inbred) to 2.297 µg/g in Golden Bantam (yellow sweet corn cultivar), respectively. While, α-carotene and β-carotene concentrations ranged from 0.332 µg/g in Country Gentleman (white sweet corn inbred) to 2.218µg/g in inbred 2 (yellow field corn inbred) and from 0.112µg/g in inbred 9 (white sweet corn inbred) to 1.801µg/g in inbred 2 (yellow field corn inbred). That to say, α- and β-carotene contents were higher in field corn genotypes than sweet corn genotypes.

The three F_1 field-sweet corn crosses showed significant variations of the carotenoids contents specially, α-carotene and β-carotene. F_1 plants of (P_{12}xP_{20}) cross with yellow mature ear had the highest concentration of zeaxanthin and β-cryptoxanthin but the lowest concentration of α-carotene and β-carotene as shown in Table 2. On the other hand, F_1 plants of (P_{16}xP_{19}) cross with yellow mature ear and (P_{12}xP_9) cross with white mature ear accumulated much more α-carotene and β-carotene. A chromatogram of the HPLC analysis of F_1 plants of (P_{16}xP_{19}) is shown in Fig. 2.

Correlation coefficients among kernel color and carotenoids content

The comparison between Hunter-b* color estimates of immature-kernels and corn-flour and β-carotene content showed that there is a poor correlation coefficient values (Table 3). On the other hand, zeaxanthin and β-cryptoxanthin significantly correlated with flour Hunter b*value. Values of b*-reading referred to the concentration of color, the higher the values, the greater pigments content. Interestingly, β-cryptoxanthin showed significant correlation with zeaxanthin while, β-carotene showed significant correlation with α-carotene (Table 3).

Mean analysis for flour yellowness index trait

Some genetic parameters of flour yellowness index were estimated using observed generation mean analysis in two sweet-field corn crosses; F_1 of (P_{16}xP_{19}) and F_1 of (P_{12}xP_{20}). The F_1 values in both crosses were less than the mid-parent values, with a tendency for the segregation towards the low parent. This was confirmed by the estimated potency values (Table 4). The significant differences between observed and calculated arithmetic means of the F_2 in the cross between P_{19} x P_{16} suggested that non-additive gene effects controlling the differences of yellowness index. This was confirmed by the estimates of F_2 geometric means, whereas no significant differences

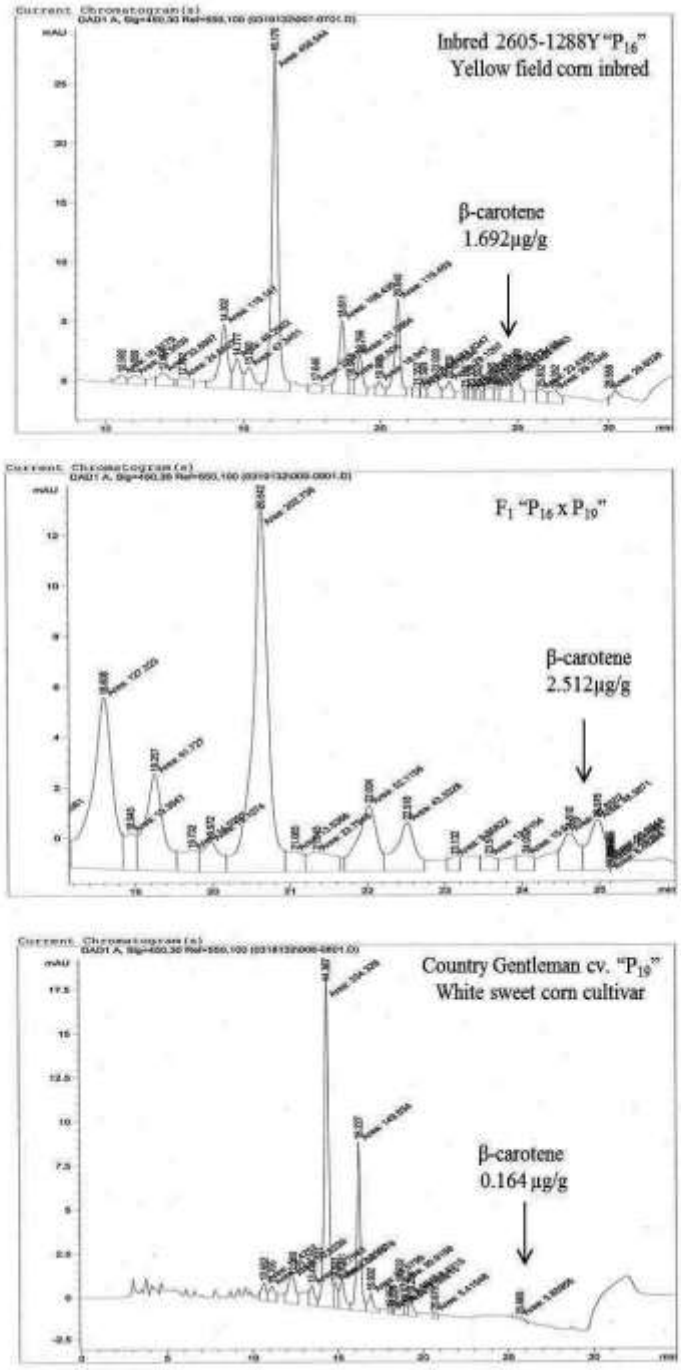

Fig. 2. HPLC analysis of β-carotene content in F_1 of field-sweet corn cross; inbred 2605-1288Y (yellow field corn inbred "P_{16}") x Country Gentleman cv. (white sweet corn cultivar "P_{19}").

Table 3. Phenotypic correlation coefficients among Hunter b*color estimates of immature-kernels and corn-flour as well as carotenoids yellow pigments contents in field-sweet corn crosses.

Traits	Immature-Kernels Hunter b*value	Flour Hunter b*value	Flour yellowness index	Zeaxanthin	β-cryptoxanthin	α-carotene	β-carotene
Immature-Kernels Hunter b*value	-	**0.639****	**0.683****	**-0.132^{ns}**	**-0.403^{ns}**	**0.041^{ns}**	**-0.137^{ns}**
Flour Hunter b*value	-	-	**0.928****	**-0.477***	**-0.586****	**-0.120^{ns}**	**-0.165^{ns}**
Flour yellowness index	-	-	-	**-0.337^{ns}**	**-0.389^{ns}**	**-0.058^{ns}**	**-0.249^{ns}**
Zeaxanthin	-	-	-	-	**0.695****	**0.085^{ns}**	**-0.061^{ns}**
β-cryptoxanthin	-	-	-	-	-	**0.311^{ns}**	**0.175^{ns}**
α-carotene	-	-	-	-	-	-	**0.868****
β-carotene	-	-	-	-	-	-	-

Ns, * and ** = not significant, significant (P≤0.05) and highly significant (P≤0.01), respectively.

were detected between the estimated and observed F_2 mean in this cross (Table 4). Values of A, B and C scaling test in this cross were insignificant, suggesting significant contribution of additive genetic effects. The additive (d) and dominance (h) genetic variance estimates for yellowness index were negative. This indicates that the three-parameter model was not adequate to explain the mode of yellowness index inheritance in both crosses. Results of the six parameter model showed that (m) values of both crosses were significant, indicating that this character inherited quantitatively. Also, the opposite sign of (h) and (l) in both crosses suggested duplicate type of genetic effects controlled the expression of this trait.

This is evidence for the presence of digenic or higher-order interactions of epistatic effect. Regardless the values sign, magnitude of (h) was higher than (l), (i), (d) and (j) in the first cross ($P_{19}xP_{16}$), but in the second cross ($P_{12}xP_{20}$), (j) values were the highest followed by (i), (d), (l) and (h) in descending order. Thus, the development of sweet-field corn genotypes having desirable yellowness index will be complicated by inherent dominance and epistatic effects. Estimates of heritability in broad-sense for yellowness index were 76.93% in cross ($P_{19}xP_{16}$) and 54.50% in cross ($P_{12}xP_{20}$). The narrow-sense heritability estimates were lower than broad-sense indicating the presence of non-additive gene action. The estimates of narrow-sense heritability were moderate in both crosses, which were 45.95% in the first cross ($P_{19}xP_{16}$) and 49.56% in the second one ($P_{12}xP_{20}$). The estimates of narrow-sense heritability demonstrated that additive genetic effects contributed to the observed yellowness index.

Table 4. Estimates of some genetic parameters using observed generation mean analysis for flour yellowness index trait in two field-sweet corn crosses.

Genetic Parameters	Inbred 2605-1288y (P_{16}) X Country Gentleman (P_{19})			Inbred 82 (P_{12}) X Golden Bantam (P_{20})		
	Observed	Expected	Difference	Observed	Expected	Difference
P_1	48.95±0.96			54.77±0.79		
P_2	28.11±0.87			20.21±0.41		
F_1	33.93±0.85	38.53	-4.60**	25.65±0.94	37.49	-11.84**
F_2	38.41±0.93	36.23	2.18**	25.60±0.50	31.57	-5.97**
Bep 1	31.45±1.10	31.02	0.43ns	23.91±0.56	22.93	0.98ns
Bep 2	39.78±0.67	41.44	1.66**	31.69±0.42	40.21	-8.52**
Gene effects						
Geometric F_2 man		35.48**			29.22**	
Potency ratio		-0.44			-0.69	
Scaling test scales						
(A)		0.85ns			1.95ns	
(B)		-3.32ns			-17.06	
(C)		8.73ns			-23.88**	
Three parameter model						
Mid-parent (m)		49.73ns			28.71ns	
Additive (d)		-10.42**			-17.28**	
Dominance (h)		- 29.47ns			-9.38ns	
Six parameter mode						
Mid-parent (m)		38.41**			25.60**	
Additive (d)		- 8.37ns			-7.78ns	
Dominance (h)		- 15.80ns			-3.06ns	
Additive x Additive (i)		11.20ns			8.78ns	
Additive x Dominance (j)		2.08ns			9.50ns	
Dominance x Dominance (l)		13.67ns			6.32ns	
Heritability						
Broad sense		76.93			54.50	
Narrow sense		45.95			49.56	

Ns, * and ** = not significant, significant (P≤0.05) and highly significant (P≤0.01), respectively.

Genotyping for lcyE polymorphisms

lcyE characterization of the field and sweet corn genotypes and field-sweet corn crosses using the specific primer of *lcyE* 3'indel (144 bp) was done and gel electrophorese is shown in Figure 3. In checking for polymorphisms of the tested genotypes, three regions were selected and scored for higher β-carotene content. The polymerase chain reaction (PCR) assay is used to survey the high and the low β-carotene genotypes. The primer *lcyE* 3'indel (Tm-59.9^{o}C) gets amplification at 144 bp. The presence of the band indicates the higher β-carotene levels and its mere absence indicates lower levels of β-carotene. Moreover, the natural mutations like 5' the large promoter insertion and 3'8-bp insertion are expected to increase the β-carotene levels. Functional polymorphisms of *lcyE* across the tested genotypes resulted in statistically significant differences either for α- or β-branch carotenoids concentration (Table 2 and Fig. 3). These genomics regions were amplified (144 bp of 3' indels) in the currently studied genotypes with higher levels of β-carotene. In field corn genotypes, five genotypes could amplify (144 bp of 3' indels); inbred1, inbred 2, inbred 6, inbred 82 (P_{12}) and inbred 2605-1288y (P_{16}). These inbreds showed the highest β-carotene accumulation. While in sweet corn, all tested genotypes did not amplify that region and had the lowest β-carotene accumulation. The two F_1 field-sweet corn crosses; (P_{12}xP_9) and (P_{16}xP_{19}) which showed high β-carotene content got amplification of *lcyE* 3'indel at 144 bp.

Discussion

There seems to be a universal interest in carotenoids biofortification, understandably in much of the world where macular degeneration and blindness can be common as a result of Vitamin A deficiency. Egypt has a strong history for introducing and successful adapting new crop species and historically field corn is a stable food of millions of people. On the other hand, sweet corn which resulted from a mutant in chromosome 4 of field corn appeared in 1779 (Poole 1937). Sweet corn is a new crop in Egypt and it needs to be improved. Sweet corn is cultivated by small-scale farmers in small areas in the northern part of Egypt around the big cities (Shaban 2003 and Mahmoud and Soliman 2007). Production from sweet-field corn hybrids would be a great way of improving income and stimulating on farm conservation as well as nutritional value. Field corn was used to improve the agronomic performance of sweet corn in USA (Tracy 1994) and in Europe (Malvar *et al* 2001). In previous studies of the author, quantitative inheritance of total soluble solids and flour color in sweet-field corn crosses was estimated (Wahba *et al* 2015). On the same time, heterosis in hybrids between sweet and field types of corn were investigated (Wahba *et al* 2016). Meanwhile in the current study, field corn was used to improve sweet corn

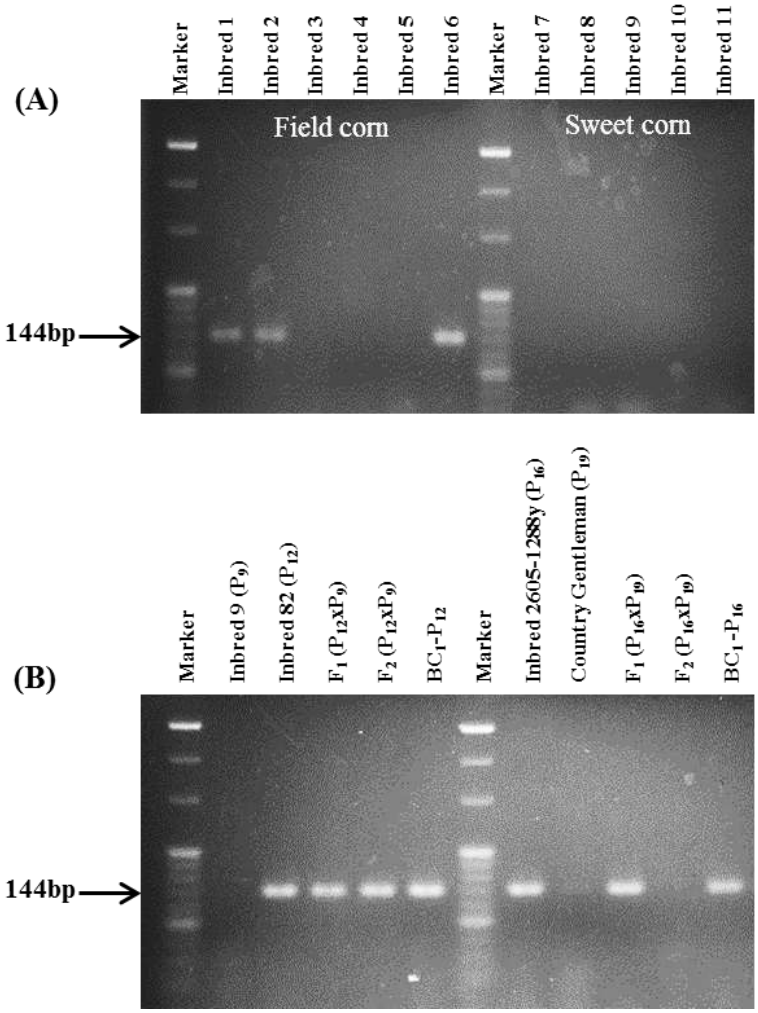

Fig. 3. Lycopene epsilon cyclase (*lcyE*) characterization of the field, sweet corn genotypes and field-sweet corn crosses using the specific primer of *lcyE* 3'indel (144 bp). (A) six Egyptian yellow field corn (*Zea mays* var. *indurata*) inbreds 1 to 6 and five Egyptian sweet corn (*Zea mays* L. var. *rugosa* Bonaf) inbreds 7 to 11. (B) two field-sweet corn crosses; inbred 9 (white sweet corn inbred "P_9") x inbred 82 (white field corn inbred "P_{12}") and inbred 2605-1288Y (yellow field corn inbred "P_{16}") x Country Gentleman cv. (while sweet corn cultivar "P_{19}"). All PCR reaction products were electrophoresed in a 4% agarose gel, stained with ethidium bromide, and visualized under UV light.

crop, especially carotenoids contents by incorporating *lycE* gene in field-sweet corn crosses in Egypt.

Carotenoids are important as sources of vitamin "A". In general, the major carotene in kernels is β-carotene but the most abundant are lutein and zeaxanthin. The appearance of mature kernels of corn can range from white to almost black. The carotenoids are primarily associated with the yellow color of the kernels (Dooner and Nelson 1979 and Dooner *et al* 1991). However, Harjes *et al* (2008) compared between β-carotene and total carotenoids with grain color and found poor correlations with low R^2 values, which indicated that using biotechnology such as marker-assisted selection (MAS) may prove much more efficient than selection based on color alone. In ongoing studies, attempts were done generally to improve sweet corn by incorporating of β-carotene lycopene epsilon cyclase (*lcyE*) gene from field corn into sweet corn for sustainable sweet corn nutritional value and production in Egypt. Hunter b*values and their genetic parameters for immature kernels and corn flour as well as carotenoids contents were investigated. *lcyE* which had the largest effect on partitioning the two branches of carotenoids (Fig.1) and consequently, on β-carotene content (Cunningham *et al* 1996 and Matthews and Wurtzel 2007) was characterized in field and sweet types of corn and field-sweet corn crosses.

In general, significant differences were observed for kernel carotenoid contents. The predominant carotenoids in kernels, as descending order of concentration, are zeaxanthin, α-carotene, β-cryptoxanthin and β-carotene (Table 2). Field corn genotypes were higher than sweet corn genotypes. However, F_1 field-sweet corn cross ($P_{12}xP_9$) accumulated as much as 3.265 µg/g β-carotene. β-carotene has two provitamin "A" structures (Harjes *et al* 2008 and Gonzalez-Jorge *et al* 2013). These results led to emphasize on increasing sweet corn carotenoid through crossing with field corn. Also, the results of heritability confirmed that good progress could be made in a program to improve the yellowness index in sweet-field corn population.

Comparisons between β-carotene and Hunter b*values for immature kernels and corn flour revealed poor correlations which in agreement with Harjes *et al* (2008). Combining information about carotenoid pathways with natural variation for carotenoids in maize grain identified several genotypes of the gene encoding (*lcyE*) that substantially increased β-carotene contents in grain (Fig.3). This combination concluded that genetic variation at this locus significantly altered the ratio of the branches, leading to increase β-carotene. Supporting evidence from QTL experiment, carotenoid QTL analysis and a mutagenesis study all pointed to *LcyE* as the causal factor for the modification of substrate flux. These results allowed the design of PCR-based markers targeted to the three polymorphisms which are currently

being used in pro-vitamin "A" breeding programs (Harjes *et al* 2008 and Safawo *et al* 2010).

CONCLUSION

In conclusion, sweet corn can be improved by using field corn. Non-significant correlation between grain color and β-carotene was observed. Functional polymorphism of *LcyE* among genotypes showed significant results along with β-carotene contents. Tested sweet corn genotypes could not amplify *LcyE* and had very low β-carotene while, field corn inbred which got the amplification of *LcyE* had high concentration of β-carotene. Field-sweet corn crosses could amplify *LcyE* and one of them gave the highest concentrations of β-carotene. It means *LcyE* genes could be incorporated from field corn into sweet corn. These results will need to be coordinated with comprehensive breeding to realize the potential of provitamin "A" biofortified field-sweet corn crosses.

ACKNOWLEDGEMENT

The author is thankful to Dr. Seifelnasr H. GadElhak, Professor of vegetables breeding, Horticulture Department, Faculty of Agriculture, Minia University, El-Minia, Egypt for providing the plant materials. Author also would like to thank Dr. David Rao, Department of Agriculture (USDA), Agricultural Research Service (ARS), Beltsville Human Nutrition Research Center, Food Components and Health Laboratory, Beltsville, MD, USA for technical advice regarding HPLC analysis of corn carotenoids. Great thanks to the Cultural Affairs & Missions, Ministry of Higher Education, Egypt for the award of Post-doctoral Fellowship, and to the Laboratory of Plant Breeding, Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan for the invitation as Visiting Scientist. Author is also thankful to the U.S. Department of USDA-ARS, Plant Sciences Institute, Genetic Improvement of Fruits and Vegetables Laboratory, Beltsville, MD, USA for the invitation as Visiting Scientist.

REFERENCES

Azizi, F., A.M. Rezai and G. Saeidi (2006). Generation mean analysis to estimate genetic parameters for different traits in two crosses of corn inbred line at three planting densities. J. Agr. Sci. Tech. 8(2):153-169.

Bouis, H.E. (2003). Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? Proceed. Nutr. Soc. 62(2):403-411.

Bouis, H.E., C. Hotz, B. McClafferty, J.V. Meenakshi and W.H. Pfeiffer (2011). Biofortification: A new tool to reduce micronutrient malnutrition. Food and Nutrition Bulletin 32 (Supplement 1):31-40.

Cavalli, L.L. (1952). An analysis of linkage in quantitative inheritance, pp. 135-144. In E.C.R. Reeve and C.H. Waddington (eds.), Quantitative Inheritance. HM Stationary Office, London, U.K.

Cazzonelli, C.I. and B.J. Pogson (2010). Source to sink: regulation of carotenoid biosynthesis in plants. Trends in Plant Science 15(5):266–274.

Cunningham, F.X.Jr., B. Pogson, Z. Sun, K.A. McDonald, D. DellaPenna and E. Gantt (1996). Functional analysis of the beta and epsilon lycopene cyclase enzymes of *Arabidopsis* reveals a mechanism for control of cyclic carotenoid formation. Plant Cell 8(9):1613-1626

Dooner, H.K. and O.E. Nelson (1979). Interaction among C, R and Vp in the control of the Bz glucosyltransferase during endosperm development in maize. Genetics 91(2):309-315.

Dooner, H.K., T.P. Robbins and R.A. Jorgensen (1991). Genetic and developmental control of anthocyanin, Ann. Rev. Genet. 25:173-199.

Farnham, D.E., G.O. Benson and R.B. Pearce (2003). Corn perspective and culture Chapter1 pp.1-33. In: PJ. White, LA Johnon, eds. Corn: Chemistry and Technology, Edition 2nd. American Association of Cerial chemicals, Inc. St. panl, Minesota, USA.

Gomez, K.W. and A.A. Gomez (1984). Statistical Procedures for Agriculture Research. John Wiley and Sone, Inc., New York.

Gonzalez-Jorge, S., S.H. Ha, M. Magallanes-Lundback, L.U. Gilliland, A.L. Zhou, A.E. Lipka, Y.N. Nguyen, R. Angelovici, H. Lin, J. Cepela, H. Little, C.R. Buell, M.A. Gore and D. Dellapenna (2013). Carotenoid cleavage dioxygenase4 is a negative regulator of β-carotene content in *Arabidopsis* seeds. Plant Cell 25(12):4812-4826.

Harjes, C.E., T. Rocheford, B. Ling, T.P. Brutnell, C.B. Kandianis, S.G. Sowinksi, S.R. Vallabheni, M. Williams, E.T. Wurtzel, J. Yan and E.S. Buckler (2008). Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. Science 18(319):330-333.

HarvestPlus (2004). Breeding crops for better nutrition /http://www.harvestplus.org/S, accessed 4 January.

Hayman, B.I. (1958). The separation of epistatic from additive and dominance in gene ratio means. Heredity 12:371-390.

Howitt, C.A. and B.J. Pogson (2006). Carotenoid accumulation and function in seeds and non-green tissues. Plant Cell Environ. 29(3):435-445.

Hulshof, P.J.M., T. Kosmeijer-Schuil, C.E. West and P.C.H. Hollman (2007). Quick screening of maize kernels for provitamin A content. Journal of Food Composition and Analysis 20(8):655-661.

Mahmoud, M.R. and T.H.I. Soliman (2007). Influence of sweet corn cultivars and plant spacings on vegetative growth, yield, quality and chemical composition characteristics in newly reclaimed soils. J. Agric. & Env. Sci. Alex. Univ., Egypt 6(2):90-116.

Malvar, R.A., P. Revilla Temiño, M.E. Cartea González, A.M. Butrón Gómez and A. Ordás Pérez (2001). Checking performance of field corn inbreds as donors of favorable alleles to improve early vigor and adaptation of sweet corn hybrids to European conditions. Maydica 46(3):187-193.

Mather, K. (1949). The genetical theory of continuous variation. Hereditas 35(S1): 376-4010.

Mather, K. and J.L. Jinks (1971). Biometrical Genetics, 2nd edition. Chapman and Hall, London.

Mather, K. and J.L. Jinks (1977). Introduction to Biometrical Genetics (No. QH430. M37 1977). London: Chapman and Hall.

Matthews, P.D. and E.T. Wurtzel (2007). Biotechnology of food colorant production. 347–398. Socaciu C., editor, Food Colorants: Chemical and Functional Properties. CRC Press, Boca Raton, FL, USA.

Moran, N.A. and T. Jarvik (2010). Lateral transfer of genes from fungi underlies carotenoid production in aphids. Science 328: 624-627.

Owens, B.F., A.E. Lipka, M. Magallanes-Lundback, T. Tiede, C.H. Diepenbrock, C.B. Kandianis, E. Kim, J. Cepela, M. Mateos-Hernandez, C.R. Buell and E.S. Buckler (2014). A foundation for provitamin A biofortification of maize: Genome-wide association and genomic prediction models of carotenoid levels. Genetics 198(4):1699-1716.

Poole, C.F. (1937). Improvement of sweet corn. USA Dept. Agric. Yearbook.

Robinson, H.F., R.E. Comstock and P.H. Harvey (1949). Estimates of heritability and the degree of dominance in corn. Agron. J. 41(8): 353-359.

Rubatzky, V.E. and M. Yamaguchi (1997). Sweet Corn, *Zea mays* L. In World Vegetables, pp. 235-252. Springer USA.

Safawo, T., N. Senthil, M. Raveendran, S. Vellaikumar, K.N. Ganesan, G. Nallathambi, S. Saranya, V.G. Shobhana, B. Abirami and E. Vijaya Gowri (2010). Exploitation of natural variability in maize for β-carotene content using HPLC and gene specific markers. Electronic Journal of Plant Breeding 1(4): 548-555.

Shaban, M.S. (2003). Genetic studies on some economic characters in sweet corn (*Zea mays* L.). M.Sc. Fac. Agric., El Moshtoher Univ., Zagazig, Egypt.

Tracy, W.F. (1994). Sweet corn. In: Specialty Corns. CRC. Press Inc. Ames, Iowa, USA.

Tracy, W.F. (1997). History, genetics, and breeding of super sweet (shrunken2) sweet corn. Plant Breeding Reviews 14:189-236.

Wahba, B.K. (2009). Genetic studies on *Zea mays* var. *Saccharata*. M.Sc. Thesis in Agric. Sci., Vegetable Crops, Fac. of Agric., Minia Univ., El-Minia, Egypt.

Wahba, B.K., H.E.M. Zaki, Y.M.M. Moustafa, Y.Y. Abdel Ati and S.H. Gadelhak (2015). Quantitative inheritance of total soluble solids and flour color in sweet-field corn crosses. Nature and Science 13(12):137-145.

Wahba, B.K., H.E.M. Zaki, Y.M.M. Moustafa, Y.Y. Abdel Ati and S.H. Gadelhak (2016). Heterosis in hybrids between sweet and field types of corn. Egypt. J. Plant Breed. 20(2):295-316.

Welch, R.M. and R.D. Graham (2002). Breeding crops for enhanced mineral content. Plant and Soil 245(1):205-214.

West, K.P. Jr. (2003). Vitamin A deficiency disorders in children and women. Food Nutr. Bull. 24(4):78–90.

تحسين محتوى الذرة الحلوة من البيتا كاروتين باستخدام الذرة الشامية في مصر

هيثم الهام محمد زكي

قسم البساتين-كلية الزراعة-جامعة المنيا-المنيا-مصر

*لوحظ وجود اختلافات معنوية لصفة لون دقيق الحبوب المطحونه "Kernel flour color" ووراثة القراءات اللونية*Hunter b بين التراكيب الوراثية التي تم تقييمها وهي٧ تراكيب وراثية من الذرة الحلوة، ٨ تراكيب وراثية من الذرة الحقلية وثلاثة هجن بين الذرة الحلوة والذرة الحقلية. وكانت قيم الـ Flour yellowness index بين نباتات الجيل الاول F_1 تقع بين متوسط الأبوين عند التهجين بين السلالة ٢٦٠٥-Y١٢٨٨ (ذرة شامة صفراء "P_{16}") وصنفCountry Gentleman (ذرة حلوة بيضاء "P_{19}") بينما انخفضت القيم عن الأب الأقل في نباتات الجيل الاول F_1 عند التهجين بين السلالة ٨٢ (ذرة شامة بيضاء "P_{12}") و وصنف Country Gentleman (P_{19}) ولكن كانت أعلى من الاب الافضل عند التهجين بين السلالة ٨٢ (P_{12}) وصنف Golden Bantam (ذرة حلوة صفراء "P_{20} "). واظهرت نتائج تحليل الكاروتينات وجود اختلافات معنوية بين محتوي الهجن من الالفا والبيتا كاروتين. حيث أظهرت نباتات الجيل الاولF_1 ما بين الاباء ($P_{12}xP_{20}$) اعلي محتوي من الزيازانثين zeaxanthin والبيتا كريبت زانثينcryptoxanthin- β في حين كانت نباتات الجيل الاول F_1ما بين الاباء ($P_{16}xP_{19}$) والاباء ($P_{12}xP_9$) اعلي في محتواها من الالفا والبيتا كاروتين. هذا وقد تمت دراسة وراثة الـ Flour yellowness index في الاباء و نباتات الجيل الاولF_1 ونباتات الجيل الثاني F_2 والتهجينات الرجعية BC للهجين ($P_{16}xP_{19}$) والهجين ($P_{12}xP_{20}$). ووجد تفاعل جينى غير أليلى بالإضافة الى التأثير المضيف للتحكم في هذه الصفة وكان التأثير الاكبر للتفاعل غير المضيف. كما تضمنت وراثة هذه الصفة ايضا" سيادة جزئية نحو الاب الاقل مع ارتفاع قيم كفاءة التوريث بالمعنى الواسع"Broad sense" والضيق "Narrow sense" لهذه الصفة Flour yellowness index . هذا واوضحت النتائج وجود ارتباط غير معنويا بين درجة لون الحبوب ومحتوي الحبوب من البيتا كاروتين، بينما كان هناك ارتباط معنوي بين وجود الجين lcyE في التراكيب الوراثية المختلفة و محتوي الحبوب من البيتا كاروتين. حيث احتوي الهجين ($P_{16}xP_{19}$) والهجين ($P_{12}xP_9$) علي هذا الجين وكانا هما الاعلي محتوي من البيتا كاروتين. هذا وتعتبر النتائج المتحصل عليها في هذه الدراسة خطوة نحو انتاج ذرة حلوة اكثر قيمة غذائية واكثر تأقلما في مصر.*

المجلة المصرية لتربية النبات ٢٠ (٥) :٧٠٥- ٧٢٢ (٢٠١٦)