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Research Article

Screening of the Romanian maize (*Zea mays* L.) germplasm for *crtRB1* and *lcyE* alleles enhancing the provitamin A concentration in endosperm

Ioan BĂCILĂ[1], Voichița HAȘ[2], Dana ȘUTEU[4]*, Mihai MICLĂUȘ[1,4], Ana COSTE[1], Edward MUNTEAN[2,3], Carmen D. VANA[2], Andrei VARGA[2], Roxana CĂLUGĂR[2], Ana COPÂNDEAN[2]

[1]*Institute of Biological Research, Branch of the National Institute of Research and Development for Biological Sciences, Department of Experimental Biology and Biochemistry, 48 Republicii Street, 400015 Cluj-Napoca, Romania; ioan.bacila@icbcluj.ro; mihai.miclaus@icbcluj.ro; ana.coste@icbcluj.ro*
[2]*Agricultural Research and Development Station, Turda, Romania; hasvoichita@yahoo.com; carmend.vana@yahoo.com; andrei_varga06@yahoo.com; roxasut@yahoo.com; copandean_ana@yahoo.com*
[3]*University of Agricultural Sciences and Veterinary Medicine, 3-5 Mănăștur Road, 400372 Cluj-Napoca, Romania; emuntean@usamvcluj.ro*
[4]*Babeș-Bolyai University, 1 Mihail Kogălniceanu Street, 400347 Cluj-Napoca, Romania; mihai.miclaus@ubbcluj.ro; dana.suteu@icbcluj.ro (*corresponding author)*

Abstract

Maize occupies a significant place in the world agriculture. Yellow kernel maize contains mainly non-provitamin A carotenoids: lutein and zeaxanthin. The accumulation of provitamin A carotenoids is regulated by favourable alleles of lcyE and crtRB1 genes and could be used for the enhancement of these carotenoids in the maize grain through breeding. In this study, molecular screening of the Romanian germplasm was performed, looking for favourable alleles of the crtRB1 and lcyE genes, and the level of carotenoids was determined in a few selected lines. A number of 2746 inbred lines from seven research stations were subjected to a PCR amplification of crtRB1 and lcyE genes in order to identify the favourable alleles. It was selected 27 lines carrying the favourable alleles and nine lines with unfavourable alleles (four groups in total), from which total carotenoids, lutein, zeaxanthin, β-cryptoxanthin, β-carotene and retinol equivalents were determined by HPLC. Out of 2746 inbred lines analysed, 23.53% contained one or both genes with favourable alleles. The favourable allele of the crtRB1 gene was the most widespread (584 lines), followed by the lcyE gene (55 lines), while alleles favourable for both genes were detected in only 7 lines. Inbred lines with the favourable allele of the crtRB1 gene showed the highest levels of β-carotene and β-cryptoxanthin, while those with favourable allele of lcyE gene showed a high level of β-cryptoxanthin; the lines with favourable alleles for both genes had a level of β-carotene 60% higher than the lines with two unfavourable alleles.

Keywords: crtRB1; lcyE; maize; provitamins A

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Introduction

Among cereals, maize (*Zea mays* L., Poaceae family) occupies a significant place in the world agriculture, providing around 30% of the food calories to 4.5 billion people of 94 developing countries together with other cereals (Shiferaw *et al*., 2011). It is known that maize yellow kernel contains carotenoids, predominantly lutein and zeaxanthin, which constitute the non-provitamin A fraction (Duo *et al.,* 2021). In contrast, the quantity of provitamin A carotenoids ranges merely from 0.25 to 2.5 mg/kg, which is insufficient to meet the prescribed daily requirement of the human beings (Tanumihardjo, 2011; Pixley *et al*., 2013; Zunjare *et al*., 2018). Based on the factors such as bioavailability ratio (of 12:1), retention up to 50% after storage/processing, level of nutrients in the host, food matrix and food consumed in the meal, HarvestPlus, a plan of CGIAR (Consultative Group on International Agricultural Research), has fixed a target of 15 mg/kg provitamin A per unit of dry weight of maize kernel for developing hybrids with high concentrations of provitamin A (Owens *et al*., 2014; Menkir *et al*. 2021). In order to meet this target, researchers have been pursuing development of provitamin A-rich maize hybrids through different approaches of genetic enhancement.

Among all cereal grains, maize exhibits tremendous natural variation for the content of provitamin A carotenoids. The carotenoid biosynthesis pathway has two major branches: α-branch and β-branch that split after the biosynthesis of linear all-trans-lycopene (DellaPenna *et al*., 2006; Menkir *et al*., 2008; Burt *et al*., 2011). Thereafter, lycopene may be cyclised either to form two β rings, as found in β-carotene and its derivatives, or to form one β ring and one ε ring, as found in α-carotene and its derivatives. The major provitamin A carotenoids in maize (β-carotene and β-cryptoxanthin) are produced in the β branch, whereas α-carotene is produced in the α-branch of the carotenoid biosynthesis pathway (Vallabhaneni *et al*., 2009).

Two genes are significant for the provitamin A carotenoid accumulation in the maize kernel: lcyE and crtRB1. LcyE gene, mapped to chromosome 8 and consisting of ten exons, alters flux down α-carotene versus the β-carotene branch of the carotenoid biosynthesis pathway (Harjes *et al*., 2008). The favourable allele of lcyE 5′TE [Transposable Element; in 5′-untranslated regions (UTR)] causes up to a 30% reduction in the ratio of α- to β-branch carotenoids and an increase in provitamin A content (Babu *et al*., 2013). The second important gene involved in this process is crtRB1, which is mapped on chromosome 10 and encodes β-carotene hydroxylase enzyme. CrtRB1 catalyses the conversion of β-carotene into β-cryptoxanthin and that of β-cryptoxanthin into zeaxanthin. By association mapping, three polymorphisms of crtRB1 viz., 5′TE (in the 5′UTR), InDel4 (in the coding region) and 3′TE (spanning the sixth exon and 3′UTR) limit the conversion of β-carotene into further components and lead to many fold increase in provitamin A concentration of maize kernel. The 3'TE polymorphism of crtRB1 gene has three alleles: allele 1 (543 bp; without TE insertion), allele 2 (296 bp + 875 bp; with 325 bp TE insertion) and allele 3 (296 bp + 1221 bp + 1250 bp; with 1250 bp TE insertion). Allele 1 is known as a favourable allele for enhancing the β-carotene content by reducing the transcript expression of the crtRB1 gene, whereas allele 2 and allele 3 cause unfavourable effects (Yan *et al*., 2010).

The significant effect of crtRB1and lcyE favourable alleles for enhanced production β-carotene in maize and accumulation of provitamin A carotenoids is now well documented and, therefore, it could be used for the enhancement of provitamin A in the maize grain by breeding (Liu *et al*., 2015; Menkir *et al*., 2017; Zunjare *et al*., 2017).

In Romania, maize is mentioned for the first time at the end of the 17th century (Pascovschi, 1957). The improvement of maize was initially empirical, people selecting the most beautiful cobs, with the largest and healthiest grains, thus leading to the development of local populations and varieties. Hybridization, as a breeding method, began by creating hybrids between varieties. The most efficient method of improving maize is by inbreeding for the creation of single crosses (hybrids) between inbred lines, elaborated by SHULL in 1908-1909 and still used in the present days.

In recent years there have been several approaches in molecular characterization of the Romanian germplasm. Thus, AFLP technique was used to determine the genetic variability existing among 60 maize landraces coming from western part of Romania (Murariu *et al*., 2019). Also, SSR markers (Şuteu *et al*., 2013) and Genotyping-by-Sequencing (GBS) techniques (manuscript in preparation) were applied for a significant number of inbred lines to decipher population structure. However, no information exists about the presence of the crtRB1 and lcyE genes and their favourable/unfavourable alleles and the variation of β-carotene content in these lines.

Thus, the objectives of this study were: *i*) - To perform a molecular screening by PCR of the Romanian germplasm looking for favourable alleles of crtRB1 and lcyE genes; *ii*) - To determine the levels of carotenoids in the identified lines using high performance liquid chromatography.

Materials and Methods

Plant material

To fulfil the goals proposed in the study, 2053 inbred lines obtained from five Romanian research stations were used: (Agricultural Research and Development Station (ARDS) Turda; National Agricultural Research and Development Institute (NARDI) Fundulea; Agricultural Research and Development Station (ARDS) Şimnic; Agricultural Research and Development Station (ARDS) Suceava; Agricultural Research and Development Station (ARDS) Lovrin). To these, almost 700 inbred lines gathered from the Institute of Crop Science "Porumbeni" Republic of Moldova and Maize Research Institute Zemun Polje, Belgrade, Serbia, were added, with the aim of obtaining a regional image of the results (Table 1).

Table 1. Distribution of inbred lines by research centres and the number of homozygous lines with favourable alleles of crtRB1, lcyE, and both crtRB1 and lcyE genes

Research stations	No. of lines	crtRB1	lcyE	Heterozygous	crtRB1+lcyE
ARDS Turda	748	191	8	66	4
NARDI Fundulea	417	79	5	15	0
ARDS Şimnic	362	56	2	14	0
ARDS Suceava	359	160	0	36	0
ARDS Lovrin	167	10	28	32	1
Rep. Moldova	592	71	7	23	0
Serbia	101	17	5	3	2
Total	**2746**	**584**	**55**	**189**	**7**

DNA isolation and PCR amplification of crtRB1 and lcyE genes

The plant material consisted of leaves from maize seedling harvested nine days after germination and dried on silica gel for 14 days. The genomic DNA was extracted using the commercial kit NucleoSpin® 96 Plant II Genomic DNA from Plant (Macherey-Nagel, Germany), according to the manufacturer's protocol, except for the final elution which took place in 80 µL (two steps) in order to increase the DNA concentration. Four random individuals were extracted twice as blind samples in each 96 samples plate (Bonin *et al*., 2004). For crtRB1-3'TE and lcyE amplifications the specific markers from Babu *et al*., (2013) were used (Table 2). The screening process targeted the favourable/unfavourable alleles of crtRB1 and lcyE genes with the following lengths: 543 bp for the favourable, and 296 bp for the unfavourable allele of the crtRB1 gene; 650 bp for the favourable, and 300 bp for the unfavourable allele of the lcyE gene.

Table 2. Sequences of primers used to amplify the crtRB1 and lcyE genes

	Sequences of primer
lcyE	Forward (F): 5'-AAG CAG GGA AGA CAT TCC AG- 3' Reverse (R): 5'-GAG AGG GAG ACG ACG AGA CAC-3'
crtRB1	Forward (F): 5'-ACA CCA CAT GGA CAA GTT CG-3', Reverse1 (R1): 5'-ACA CTC TGG CCC ATG AAC AC-3' Reverse2 (R2): 5'-ACA GCA ATA CAG GGG ACC AG-3'

30% diluted genomic DNA was used for amplification. For the crtRB1 gene, the composition of the PCR mixture was as follows: 1X MyTaq Reaction Buffer (including 1 mM dNTP, 3 mM $MgCl_2$); 0.16 µM; 0.12 µM reverse primer R1, 0.12 µM reverse primer R2, 1U polymerase MyTaq (Bioline); 1.5 µL diluted genomic DNA, PCR grade water up to a final volume of 12.5 µL. For the lcyE gene, the composition of the PCR mixture was: 1X MyTaq Reaction Buffer (including 1 mM dNTP, 3 mM $MgCl_2$); 0.12 µM forward primer; 0.12 µM reverse primer R, 1.25 U polymerase MyTaq (Bioline); 1.5 µL diluted genomic DNA, and PCR grade water up to a final volume of 12.5 µL. The PCR program used was the same for the two genes analysed: polymerase activation 5 min at 95 °C, 35 X (denaturation 95 °C for 30 sec, primer annealing 60 °C for 30 sec, elongation 72 °C for 30 sec), final elongation 5 min at 72 °C.

The amplicons were resolved on a 3.0% agarose gel and were scored for the presence or absence of allele 1 (favourable allele 543 bp) of crtRB1-3'TE gene and favourable allele (650 bp) of lcyE gene.

Determination of carotenoids content by HPLC

From the lines identified with favourable alleles, we selected 27 lines with one, two or both favourable homozygous alleles for crtRB1 and lcyE genes (three groups). To these three groups of lines, nine lines with unfavourable homozygous alleles (a fourth group) have been added for carotenoid level comparison. All the 36 lines were analysed by HPLC (high performance liquid chromatography) technique (Table 3).

Specific standards were used for lutein, zeaxanthin, β-cryptoxanthin and β-carotene (Sigma-Aldrich/Merck); HPLC grade acetonitrile, ethyl acetate and acetone (Merck, Darmstadt, Germany) and absolute ethanol for analysis (Chimreactiv, Romania).

Carotenoids were extracted from ~1 g milled grain samples with 50 mL of ethanol, on a AM4 magnetic stirrer (Velp Scientifica, Italy) for 30 minutes, the resulting suspension being then filtered on a G3 frit, under vacuum. For the quantification of major carotenoids, an aliquot from the extract was evaporated to dryness under vacuum at 40 °C in a Laborota 4010 rotary evaporator (Heidolph Instruments, Germany), then redissolved in 5 mL acetone, filtered through a 0.47 µm membrane filter and subjected to HPLC.

The determination of total carotenoids was accomplished using a T80+ UV-VIS spectrophotometer (PG Instruments Ltd., UK).

HPLC analysis was accomplished using a Flexar system (Perkin Elmer, USA), consisting in two UHPLC pumps, a solvent degasser, an autoinjector, an UV-VIS detector, a column oven, a controller and a computer running Chromera software. Separations were monitored at 450 nm, using a Nucleosil 3-C18 column (Macherey Nagel) and a gradient involving ethyl acetate (A) and a mixture of 9:1 acetonitrile: water, at 25 °C, at a flow rate of 1 mL/min (Muntean and Rotar, 2010; Muntean, 2020; Calugar *et al*., 2022). The quantitative determinations were accomplished using the external standard method, with three replicates for each sample (the mean values were reported).

Table 3. List of the 36 maize germplasm lines used for HPLC analysis

	No.	Inbred line	Research stations		No.	Inbred line	Research stations
Homozygous lines favourable for crtRB1 and lcyE genes	1	T158	ARDS Turda	Homozygous lines favourable for crtRB1 gene	19	PI2646-83	ARDS Turda
	2	T168	ARDS Turda		20	PI920-78	ARDS Turda
	3	T169a – A	ARDS Turda		21	N4	ARDS Turda
	4	T169a –R	ARDS Turda		22	PED574	ARDS Turda
Homozygous lines favourable for lcyE gene	5	TB330	ARDS Turda		23	TD302	ARDS Turda
	6	Pop.92- NS	ARDS Turda		24	PI43/97	ARDS Turda
	7	TC382	ARDS Turda		25	PED2884-11	ARDS Turda
	8	Lv6208	ARDS Lovrin		26	TB367	ARDS Turda
	9	Lv2076	ARDS Lovrin		27	TC177	ARDS Turda
	10	Lv5020	ARDS Lovrin	Homozygous lines unfavourable for crtRB1 and lcyE genes	28	TD337	ARDS Turda
	11	Lv5127	ARDS Lovrin		29	TA422	ARDS Turda
	12	Lv5004	ARDS Lovrin		30	CO255	ARDS Turda
	13	Lv5082	ARDS Lovrin		31	TA24	ARDS Turda
	14	Lv5199	ARDS Lovrin		32	PI108/71	ARDS Turda
	15	Lv2089	ARDS Lovrin		33	F1118	ARDS Turda
	16	Lv6276	ARDS Lovrin		34	RA11	ARDS Turda
Homozygous lines favourable for crtRB1 gene	17	TD339	ARDS Turda		35	TE244	ARDS Turda
	18	F91a	ARDS Turda		36	TB369	ARDS Turda

Results and Discussion

PCR screening results

Using PCR, the aim was to obtain amplicons for favourable alleles of crtRB1 (543 bp) and lcyE (650 bp) genes, and the unfavourable alleles for the two genes, 296 bp and 300 bp, respectively (Figure 1). Out of the 2746 inbred lines analysed, there were 584 inbred lines with the favourable allele for crtRB1 gene, 55 inbred lines with the favourable allele for lcyE gene, and 7 inbred lines with the favourable allele for both genes (Table 1, Figure 2, Figure 3a).

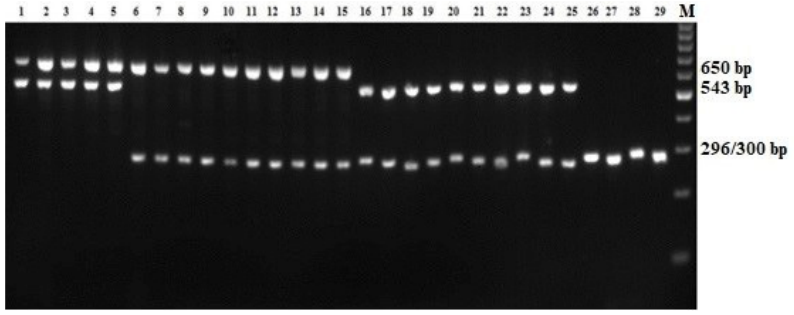

Figure 1. Pattern of allelic variation for crtRB1 3' TE and lcyE genes during a multiplex PCR for 29 inbred lines

Lanes 1-5: Inbreeds with favourable alleles (543/650 bp) for both genes, crtRb1 and lcyE; Lanes 6-15: Inbreeds with favourable allele (650 bp) for lcyE gene and unfavourable allele for crtRB1 gene (296 bp); Lanes 16-25: Inbreeds with favourable allele (543 bp) for crtRB1 gene and unfavourable allele for lcyE gene (300 bp); Lanes 26-29: Inbreeds with unfavourable alleles (296/300 bp) for both genes; M: 100 bp Ladder (Solis Biodyne)

Figure 2. Map showing the geographical locations of the research centres providing the analysed lines and the screening results obtained from each research centre: blue - homozygous lines favourable for crtRB1 gene; orange - homozygous lines favourable for lcyE gene; green - homozygous lines favourable for both crtRB1 and lcyE genes

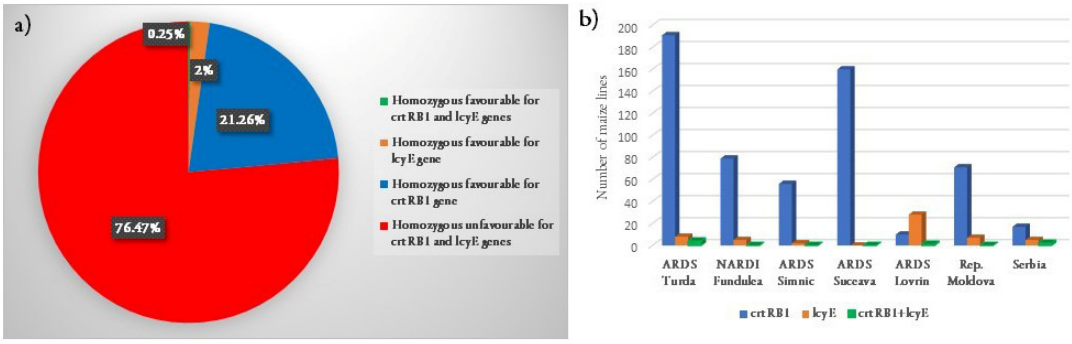

Figure 3. a) – Results of the molecular screening process performed on 2746 inbred lines: red (2100 homozygous lines unfavourable for crtRB1 and lcyE genes); blue (584 homozygous lines favourable for crtRB1 gene); orange (55 homozygous lines favourable for lcyE gene); green (7 homozygous lines favourable for crtRB1 and lcyE genes); b) - Results of the molecular screening distributed according to research stations: green (homozygous lines favourable for both crtRB1 and lcyE genes); blue (lines favourable for crtRB1); orange (homozygous lines favourable for lcyE gene)

The screening process showed that the favourable allele of the crtRB1 gene is much more common than the favourable allele of the lcyE gene, ranging from 10 lines in the case of ARDS Lovrin to 191 lines belonging to ARDS Turda. By contrast, the favourable allele of the lcyE gene is found much less frequently, from 2 lines in case of ARDS Șimnic to 28 lines from ARDS Lovrin, and missing in the case of lines coming from ARDS Suceava (Table 1, Figure 2, Figure 3b). Although a positive interaction has been observed (Azmach *et al*., 2013; Gebremeskel *et al*., 2017), favourable alleles of both genes are rare and occur infrequently in maize germplasm (1-4%) (Babu *et al*., 2013; Zunjare *et al*., 2017). We confirm these observations in our analyses, identifying only 7 lines that had favourable alleles for both genes, representing a lower percentage (0.25%) than expected (Figure 3a). A number of 189 heterozygotes were further discovered by our analyses (Table 1).

An interesting aspect is the dispersion of favourable alleles, as a clustering of lcyE favourable alleles can be observed in the southwest of the territory, similar to the neighbouring Serbia, while no occurrence was identified in the 359 analysed lines in the northern part of the territory (ARDS Suceava) (Figure 2; Figure 3b).

Our screening results underline an existing potential of the Romanian germplasm towards increasing the concentration of provitamin A through multiple possibilities of crossing between the lines from different geographical areas, having one, two or both favourable alleles for the genes of interest.

HPLC results obtained for inbred lines with the favourable allele of crtRB1 gene

We analysed 11 inbred lines with the favourable allele of crtRB1 gene by HPLC in order to determine the carotenoid content of the kernels. The concentration of β-carotene in the inbreeds ranged from 0.37 to 10.43 mg/kg, with a mean of 3.56 mg/kg. The highest value was found for the PED574 line (ARDS Turda), a line with normal yellow dent kernel. The lowest value was found for the F91a line (NARDI Fundulea) with light yellow dent kernel. The level of β-cryptoxanthin varied between 0.46 and 6.75 mg/kg, with a mean of 3.38 mg/kg (Table 4). PED574 had the highest value, whereas F91a had the lowest value.

Beta-carotene, β-cryptoxanthin and retinol equivalents levels in lines with homozygous favourable alleles for the crtRb1 gene (Figure 4e) are higher compared to the inbreeds possessing the unfavourable allele (Figure 4d, f). Similar levels were determined in the case of total carotenoids and lutein (Fig. 4a, b), while a lower level was obtained in the case of zeaxanthin (Figure 4c), suggesting that the feedback inhibition may reduce the total flux into the carotenoid pathway. Also, we could not rule out the possibility of existing nucleotide polymorphisms in the crtRB1 gene that may cause differences in gene expression, thus leading to phenotypic variations. Therefore, the influence of genetic background leading to a difference in the phenotypic expression must be accounted for (Vignesh *et al*., 2013).

Table 4. Average results were obtained by HPLC determination for the following components: total carotenoids, lutein, zeaxanthin, β-cryptoxanthin, β-carotene and retinol equivalents (RE) in different groups of inbred lines (with one or more favourable genes combination, homozygous lines unfavourable for crtRB1 and lcyE genes have been added for carotenoid level comparison)

	Total carotenoid [mg/kg]	Lutein [mg/kg]	Zeaxanthin [mg/kg]	β-cryptoxanthin [mg/kg]	β-carotene [mg/kg]	RE [mg/100g]
Homozygous favourable for crtRB1 (11 lines)	23.68	10.09	4.24	3.38	3.56	87.55
Homozygous favourable for lcyE (12 lines)	18.86	5.01	7.74	3.27	1.67	55.10
Homozygous favourable for crtRB1 and lcyE (4 lines)	17.00	2.92	7.97	2.40	2.89	68.09
Homozygous unfavourable for crtRB1 and lcyE (9 lines)	22.18	10.04	5.42	2.38	1.73	48.75

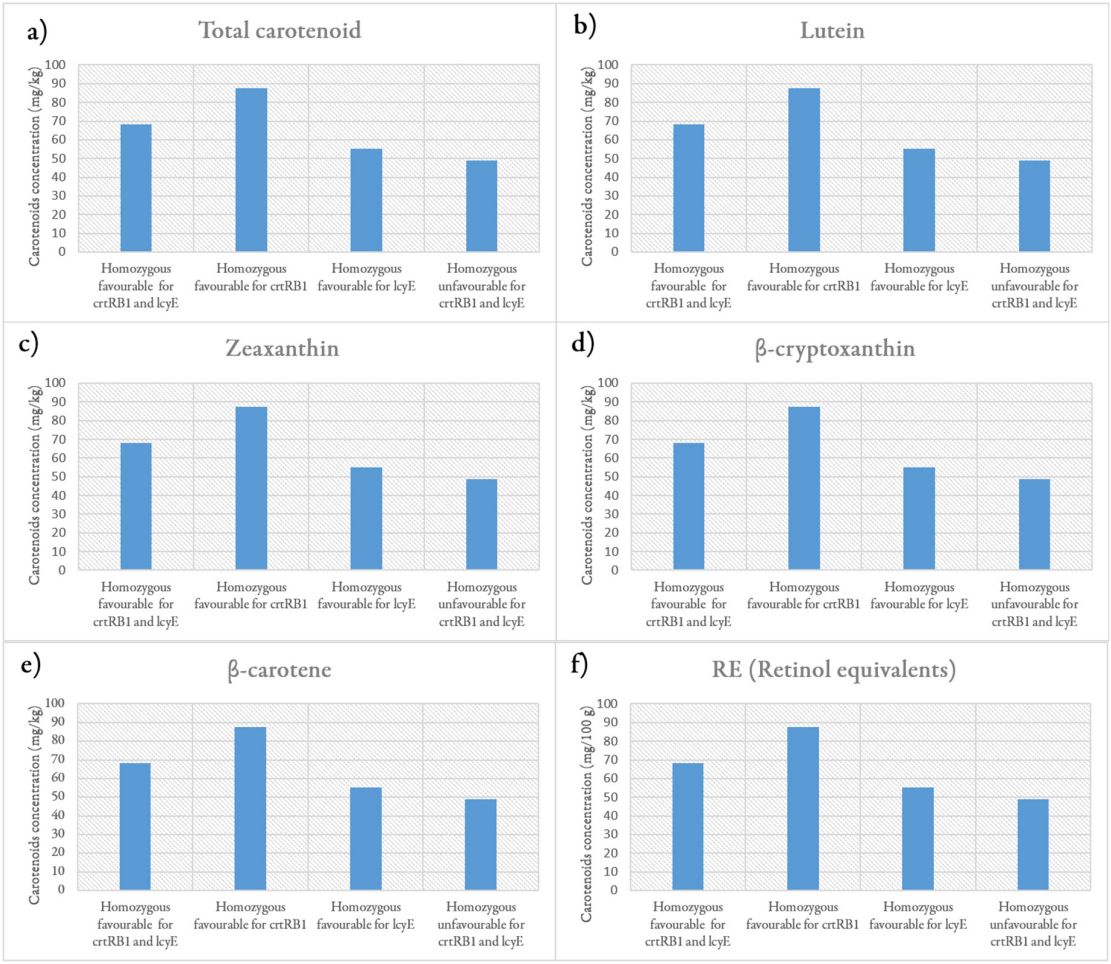

Figure 4. HPLC results (mean values) for the main categories of carotenoids in different groups of inbred lines (with one or more favourable genes combination, homozygous lines unfavourable for crtRB1 and lcyE genes have been added for carotenoids level comparison): total carotenoids (a); lutein (b); zeaxanthin (c); β-cryptoxanthin (d); β-carotene (e); retinol equivalents (f)

HPLC results obtained for lycopene epsilon cyclase (lcyE) gene

We analysed by HPLC 12 inbred lines that showed a favourable allele for the lcyE gene, to determine the carotenoid content of the kernel. The concentration of β-carotene in the inbreeds possessing the favourable allele ranged from 0.72 to 3.05 mg/kg, with a mean of 1.67 mg/kg. The highest value was found for the TC382 line (ARDS Turda), a line with dark yellow semi-dent kernel. The lowest value was found for the LV5004 line (ARDS Lovrin), a line with dark yellow dent kernel. The β-cryptoxanthin level ranged from 1.44 to 4.79 mg/kg, with a mean of 3.27 mg/kg (Table 4). Again, the highest value was found for the TC382 line and the lowest value was found for the LV5004 line. Beta-carotene, as well as total carotenoid and RE levels, in lines with favourable homozygous alleles for the lcyE gene (Figure 4a, e, f) are almost at par with the inbreeds possessing the unfavourable allele, whereas a lower level of lutein and a higher level of β-cryptoxanthin and zeaxanthin (Figure 4b, c, d) were identified in favourable homozygous alleles lines. Even though the favourable lcyE allele diverts the biosynthesis towards the α-branch, downstream in the pathway most of the carotenoids will get hydroxylated to non-provitamin A compounds, like zeaxanthin, leading to a drastic reduction of the β-carotene content in the endosperm (Vallabhaneni *et al.*, 2009). Other possible explanations for the lack of difference between the levels of β-carotene in the favourable and unfavourable lines include the carotenoid biosynthesis pathway, which is regulated by many other genes and the identified inbreeds might not possess the favourable

alleles for these (Muthusamy *et al*., 2015). The effect of the background genome might also play a role in the regulation of carotenoid biosynthesis pathway, as noticed by Babu and collaborators, who found similar results while validating the effect of lcyE 5′TE allele across five populations (Babu *et al*., 2013).

HPLC results obtained for inbred lines with favourable alleles for both genes

The four inbred lines analysed revealed higher levels of β-carotene (approximately 60% higher), zeaxanthin, β-cryptoxanthin and RE than the lines with both genes with unfavourable alleles (Figure 4c, d, e, f), but also significantly lower lutein and total carotenoid levels (Figure 4a, b).

It is important to highlight in this context that these lines are simultaneously low in lutein content (α-branch pathway) and high in zeaxanthin (β-branch pathway), suggesting once again the probability of polymorphisms present in the coding genes along the carotenoid biosynthesis chains, that may cause the difference in expression of the genes, thus producing phenotypic variations (Muthusamy *et al*., 2015). However, this hypothesis can be validated only by comparing nucleotide differences vis-à-vis estimating the accumulation of transcripts in the contrasting genotypes (Vignesh *et al*., 2013). The rather low level of total carotenoids belonging to these four lines compared to inbred lines with unfavourable alleles suggests that feedback inhibition may be reducing the total flux into the carotenoid pathway (Figure 4a). In this regard, earlier concerns that reducing the quantity of carotenoids may lead to compromised abiotic stress tolerance in crop plants have been raised (Tan *et al*., 1997).

A recent study reveals how biofortification with provitamin A can help reduce aflatoxin contamination in maize. Aflatoxins are harmful compounds that are produced by the fungus *Aspergillus flavus*, which can be found in the soil, plants and grain of a variety of legumes and cereals including maize. Suwarno and collab. (2019) demonstrate that β-carotene, β-cryptoxanthin, and provitamin A concentrations already present in biofortified hybrids can provide an advantage for reducing aflatoxin levels in maize.

According to these results, maize with increased content of provitamin A carotenoids may offer double health benefits by reducing aflatoxin concentrations while contributing to reduce vitamin A deficiency in affected maize consuming populations.

Conclusions

Out of a total of 2746 analysed inbred lines 23.53% contained one or both favourable alleles in the genes of interest, while the remaining 76.47% represented lines with unfavourable alleles. The favourable allele of the crtRB1 gene was the most widespread, occurring in 584 lines (21.26%), followed by the favourable allele of lcyE gene (55 lines representing 2%), and last, by the co-occurrence of alleles favourable for both genes (detected in only 7 lines - 0.25%). Inbred lines with a favourable allele for the crtRB1 gene showed the highest level of β-carotene and β-cryptoxanthin, while inbred lines with a favourable allele for the lcyE gene showed a high level of β-cryptoxanthin. Inbred lines with favourable alleles for both genes exhibited a 60% higher level of β-carotene than the lines with unfavourable alleles of the two genes. Results indicate that these inbred lines with enhanced carotenoid profiles hold great promise for breeding programs as well as for food and nutritional security especially in areas that are most affected by vitamin A deficiencies.

Authors' Contributions

The contributions of authors to the manuscript are as follows: conceptualization: IB; field work: VH, CDV, AV, RC, ACop.; analytical investigation: EM; data curation: EM, ACop; formal analysis: IB, DȘ, AC, MM; funding acquisition: DȘ; investigation: IB; methodology: IB; project administration: IB; writing -

original draft: IB; writing - review and editing: IB, DȘ, MM, AC, VH and EM. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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