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# β-CAROTENE CONTENT IN MAIZE GRAIN AND THE ALLELIC STATE OF THE LYCOPENE-ε-CYCLASE AND β-CAROTENE HYDROXYLASE 1 GENES IN MAIZE GENOTYPES OF UKRAINIAN BREEDING

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**Aim.** To identify potential donors of increased β-carotene content in grain and favorable alleles of the lycopene-ε-cyclase (*lyc*$_\varepsilon$) and β-carotene hydroxylase 1 (*crtRB1*) genes among maize genotypes of Ukrainian breeding by assessing the β-carotene content in grain and analyzing the associations between the β-carotene content and the allelic state of carotenogenesis genes. **Methods.** Spectrophotometric analysis, polymerase chain reaction, statistical methods. The content of total carotenoids by β-carotene in corn kernels was analyzed twice: at the time of harvest (September 2024) and after nine months of storage (June 2025). **Results.** Significant variability in β-carotene content among the studied genotypes was established. At the time of grain harvesting, this indicator was on average 4.67 mg/kg (2.70–7.14 mg/kg), and after nine months of storage, it decreased on average by 46.68% and amounted to 2.49 mg/kg (1.98–3.71 mg/kg). The degree of loss varied considerably depending on the genotype (12.59–62.18%). The two-way ANOVA analysis showed that the greatest contribution to the variation of the «β-carotene content» trait in grain was the duration of storage (51.7%), while the contribution of the genotype was 30.7%, and their interaction — 15.2%. According to the *lyc*$_\varepsilon$-SNP216 marker of the lycopene-ε-cyclase gene, the genotypes with the G allele (61.1%), the T allele (33.3%) and one heterozygous G/T genotype (5.6%) were identified, while according to the *crtRB1*-3′TE marker of the β-carotene hydroxylase 1 gene, unfavorable (296 and 296+875 bp) alleles (94.4%) prevailed. A tendency towards an increase in the β-carotene content was found in genotypes with the favorable G allele of the *lcy*$_\varepsilon$ gene (4.79±0.43 mg/kg) compared to genotypes with the T allele (4.13±0.55 mg/kg). The genotype Kros256S with the heterozygous state of *crtRB1* (296+543 bp) was characterized by an increased post-storage content of β-carotene (3.71 mg/kg). At the same time, the detection of albeit limited number of genotypes with a favorable (543 bp) allele provides an opportunity to conduct a full-fledged statistical analysis of the influence of favorable alleles of this gene on the post-storage content of β-carotene in future. It was found that the most promising potential donors for maize breeding for an increased content of provitamin A are the genotypes Kros268S and DK3172

ZM, which combined a high content of β-carotene with the presence of a favorable allele G of the $lyc_{\varepsilon}$ gene, as well as Kros256S, which was characterized by a high content of β-carotene both at the time of grain harvest and after nine months of storage and contained the favorable allele G of the $lyc_{\varepsilon}$ gene and the allele 543 bp of *crtRB1* gene. **Conclusions.** For corn genotypes of Ukrainian selection, the content of β-carotene in the grain at the time of harvesting and after nine months of storage was comprehensively assessed in combination with the determination of the allelic status of the $lcy_{\varepsilon}$ and *crtRB1* genes. The features of the manifestation of the allelic state of the $lcy_{\varepsilon}$ and *crtRB1* genes regarding the variability of the β-carotene content in the grain and its preservation were established. Potential donors of increased β-carotene content in grain and favorable alleles of carotenogenesis genes $lyc_{\varepsilon}$ and *crtRB1* were identified among the maize genotypes of Ukrainian breeding. The genotypic variability of β-carotene content in grain was assessed at the time of grain harvest and after nine months of storage. Genotypes carrying the favorable allele G of the $lyc_{\varepsilon}$ gene were characterized by a tendency to increased β-carotene content in grain, while the assessment of the influence of the favorable allele 543 bp of the *crtRB1* gene requires an expansion of the sampling of studied genotypes. Genotypes Kros268S, DK3172 ZM and Kros256S may be considered to be the most promising potential donors for maize breeding for increased β-carotene content in grain. The results obtained can be used for selection of starting material and marker-assisted selection of maize for increased β-carotene content in grain.

**Keywords.** *Zea mays* L., *lcyε*, *crtRB1*, molecular genetic markers, carotenoids, DNA polymorphism, genetic variability, provitamin A, grain storage.

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## INTRODUCTION

The β-carotene content in maize grain is an important trait determining its value as a source of provitamin A for human needs.

These factors are driving growing interest in the development of genotypes with higher β-carotene content and their introduction into production (Munkhuwa et al., 2023; Kljak et al., 2024; Menkir et al., 2025). At the same time, the level of this carotenoid is characterized by significant variability and depends on a set of factors: genotype, cultivation conditions, and grain storage conditions, etc. (Šimić et al., 2023; Denysiuk et al., 2024; Gunjević et al., 2024; Mng'ong'o et al., 2024; Saenz et al., 2025).

Genotype plays a key role in forming the variability of β-carotene content, particularly the lycopene-ε-cyclase ($lyc_{\varepsilon}$) gene and the β-carotene hydroxylase 1 (*crtRB1*) gene, whose allelic state determines both the accumulation level and the stability of this pigment in maize grain (Harjes et al., 2008; Yan et al., 2010; Băcilă et al., 2022; Sayadi Maazou et al., 2022; Bhatt et al., 2023). The $lyc_{\varepsilon}$ enzyme gene regulates the direction of the carotenoid metabolic pathway, catalyzing the asymmetric cyclization of lycopene and directing biosynthesis toward the α-branch with the formation of α-carotene, zeaxanthin, and lutein (Harjes et al., 2008; Bhatt et al., 2023). When $lyc_{\varepsilon}$ gene expression is inhibited, the metabolic pathway is redirected toward the β-branch, promoting increased synthesis of β-carotene, β-cryptoxanthin, and zeaxanthin. Thus, the level of β-carotene formation in maize grain directly depends on the allelic state of the $lyc_{\varepsilon}$ gene, whether it is favorable or unfavorable for reducing its expression and the accumulation of β-carotene.

At the same time, the elevated initial β-carotene content in maize grain after harvesting does not always remain stable during storage. This parameter is largely determined by the activity of the enzyme β-carotene hydroxylase 1 and the storage conditions. The *crtRB1* enzyme catalyzes the conversion of β-carotene to zeaxanthin, which leads to a decrease in β-carotene content (Yan et al., 2010; Bhatt et al., 2023). The presence of a favorable allele of the *crtRB1* gene, associated with decreased expression, promotes the preservation of β-carotene by limiting the hydroxylation process. It is known that maize inbreds with this allele are characterized by significantly higher levels of provitamin A and significantly lower levels of transcripts compared to inbreds carrying the wild-type allele (Bhatt et al., 2023). Consequently, the allelic state of the *crtRB1* gene determines the extent of the reduction in β-carotene content during storage and, accordingly, is an important factor in its variability during the post-harvest period.

Along with genotype, cultivation conditions have a significant influence on β-carotene content in maize grain. For instance, in the publication of D. Šimić et al. (2023), it was shown that the effect of cultivation conditions on β-carotene content was statistically significant ($P \leq 0.05$), but it was less considerable than the effect of genotype, which was highly reliable

($P \leq 0.01$), whereas the interaction between these factors had no statistically significant effect. At the same time, other studies indicate that agronomic factors, particularly the level of nitrogen fertilization, can significantly modify the β-carotene content in grain. For example, in Tanzanian genotypes grown on poor tropical soils, its content ranged from 0.38 to 13.08 mg/kg depending on the fertilizer application rate (Mng'ong'o et al., 2024).

The effectiveness of breeding improvement in maize genotypes in terms of the trait «β-carotene content in grain» is confirmed by the results of recurrent selection involving two recipient inbreds with an unfavorable allele of the *crtRB1* gene (with β-carotene content of 0.60 and 1.20 mg/kg) and a donor inbred with a favorable allele (15.20 mg/kg). As a result, genotypes with significantly increased β-carotene content up to 14.35 and 13.62 mg/kg, respectively, were obtained (Chandrasekharan et al., 2022).

Furthermore, it was found that the combination of favorable alleles of the $lyc_{\varepsilon}$ and *crtRB1* genes has a synergistic effect: the β-carotene content in such genotypes is 60% higher compared to genotypes carrying unfavorable alleles of both genes (Băcilă et al., 2022). This highlights the importance of comprehensive consideration of the allelic state of carotenogenesis genes when assessing trait variability.

Despite a significant number of studies, the relationship between the allelic state of the $lyc_{\varepsilon}$ and *crtRB1* genes and the variability in β-carotene content, especially during grain storage, remains poorly elucidated. At the same time, not only the initial level of β-carotene but also its content after long-term storage is of practical importance, since maize grain can be used both immediately after harvesting and after a certain storage period. The evaluation of the relative losses of β-carotene during storage allows for characterizing the stability of the «β-carotene content» trait, whereas the selection of promising donors for breeding should be based primarily on the absolute β-carotene content in the grain at the time of harvesting and after storage, which is associated with the allelic state of the main carotenogenesis genes.

**The aim of this study** was to identify, among maize genotypes of Ukrainian breeding, potential donors with elevated β-carotene content in grain at harvesting and after storage, as well as donors of favorable alleles of the lycopene-ε-cyclase ($lyc_{\varepsilon}$) and β-carotene hydroxylase 1 (*crtRB1*) by evaluating β-carotene content in grain and analyzing the associations between β-carotene content and the allelic state of carotenogenesis genes.

## MATERIALS AND METHODS

The study used eighteen maize genotypes of Ukrainian breeding, originally bred by the State Enterprise Institute of Grain Crops of the National Academy of Agrarian Sciences of Ukraine (Dnipro, Ukraine) and listed in the State Register of Varieties Suitable for Dissemination in Ukraine. The material under study included six hybrids and their parental forms: the DN Kyiakhy hybrid and its parental forms Kros201S and DK7400SVZM; the DN Sarmat hybrid and its parental forms DK5002M and DK633/325 MV; the DB Tyras hybrid and its parental forms, Kros160S and DK7400SVZM(2); the DN Lehenda hybrid and its parental forms, Kros268S and DK3172 ZM; the DN Halateia hybrid and its parental forms, DK315M (sterile) and DK239MV; the DN Meotyda hybrid and its parental forms, Kros256S and DK2831SV. The studied maize genotypes combine a number of valuable economic and breeding traits, including high yield, intensive moisture-yielding ability of the grain, rapid early growth, resistance to major diseases and pests, cold tolerance, and resistance to lodging.

The total carotenoid content based on β-carotene (hereinafter referred to as β-carotene content) in maize grain was analyzed twice. The first measurement was taken at the time of harvesting (September 2024), and the second one — after nine months of storage (June 2025). The grain was stored in a dark, well-ventilated room at a constant temperature of 18–22°C.

The β-carotene content was determined using a spectrophotometric method (Rodriguez-Amaya et al., 2004) at a wavelength of 450 nm, with chloroform as a solvent. For each genotype, the analysis was performed in three biological replicates. The SF-2000 spectrophotometer was used. The β-carotene content was expressed in mg/kg of grain as calculated in completely dry matter.

The allelic state of the carotenogenesis genes lycopene-ε-cyclase and β-carotene hydroxylase 1 was determined using the corresponding molecular genetic markers $lyc_{\varepsilon}$-SNP216 and *crtRB1*-3′TE (Harjes et al., 2008; Yan et al., 2010).

The polymerase chain reaction was performed under the following conditions: initial denaturation — at 94°C for 5 min, followed by 40 cycles, including denaturation (94°C, 1 min), primer annealing (60°C, 1 min), and elongation (72°C, 1 min); final elonga-

tion — 72°C for 5 min, the cooling phase — at +5°C. The amplification products were separated by horizontal electrophoresis in a 2% agarose gel.

Based on the *lyc*$_{\varepsilon}$-SNP216 marker, two allelic variants were identified on the electropherograms: the presence of a 395 bp fragment, corresponding to the allele with the nucleotide thymine at this marker site (T, the unfavorable allele), and the absence of an amplified product (null allele), corresponding to the presence of the nucleotide with the nitrogenous base, guanine (G, favorable allele) at the marker site (Harjes et al., 2008). Since the G allele is recorded as the null allele for the lcyε-SNP216 marker, duplex PCR was performed using an internal control — primers to the maize alcohol dehydrogenase gene *adh1* — to confirm the presence of DNA in the samples. Here an amplified fragment of 234 bp was observed, indicating the presence of DNA in the reaction mixture.

For the *crtRB1*-3´TE marker, alleles of 543 bp (favorable), 296 bp, and 296+875 bp (unfavorable) were registered in the electrophoregram (Yan et al., 2010; Muthusamy et al., 2015; Sagare et al., 2015; Băcilă et al., 2022).

The statistical analysis of the results was conducted using common methods (Ewens, Brumberg, 2023). The data are presented as $x \pm SE$, where $x$ is the mean value of the indicator and $SE$ is the standard error at a significance level of 0.05.

## RESULTS

The β-carotene content in maize grain was determined by both genotype characteristics and the duration of grain storage **(Table 1)**. An analysis of β-carotene content in grain of different maize genotypes revealed significant variability both at the time of harvesting and after nine months of storage. The initial β-carotene content in the studied genotypes averaged 4.67 mg/kg of grain. No statistically significant differences were found between the hybrids and their parental forms in terms of average initial β-carotene content (4.76±0.52 and 4.63±0.44 mg/kg, respectively), indicating a similar potential for pigment accumulation in both groups. The highest β-ca-

**Table 1.** β-carotene content in maize grain

| Genotype | β-carotene content at the time of harvest, mg/kg | β-carotene content after 9 months of storage, mg/kg | Decrease in β-carotene content after 9 months of storage, % |
|---|---|---|---|
| DN Kyiakhy | 2.83±0.14$^{f}$ | 1.98±0.03$^{g}$ | 30.04±0.14$^{e}$ |
| Kros201S | 3.27±0.13$^{e}$ | 2.26±0.04$^{f}$ | 30.89±0.14$^{e}$ |
| DK7400SVZM | 3.41±0.13$^{e}$ | 2.20±0.21$^{f}$ | 35.48±0.25$^{d}$ |
| DN Sarmat | 5.38±0.27$^{c}$ | 2.62±0.06$^{c}$ | 51.30±0.28$^{b}$ |
| DK5002M | 5.03±0.16$^{c}$ | 2.53±0.03$^{c}$ | 49.70±0.16$^{b}$ |
| DK633/325MV | 5.60±0.17$^{c}$ | 2.57±0.01$^{c}$ | 54.11±0.17$^{b}$ |
| DB Tyras | 6.48±0.16$^{b}$ | 3.04±0.02$^{b}$ | 53.09±0.16$^{b}$ |
| Kros160S | 4.97±0.18$^{c}$ | 2.51±0.03$^{c}$ | 49.50±0.18$^{b}$ |
| DK7400SVZM(2) | 3.81±0.08$^{d}$ | 2.46±0.06$^{d}$ | 35.43±0.10$^{d}$ |
| DN Lehenda | 4.35±0.16$^{d}$ | 2.03±0.05$^{g}$ | 53.33±0.17$^{b}$ |
| Kros268S | 7.14±0.10$^{a}$ | 2.70±0.04$^{c}$ | 62.18±0.11$^{a}$ |
| DK3172 ZM | 6.46±0.14$^{b}$ | 2.57±0.09$^{c}$ | 60.22±0.17$^{a}$ |
| DN Halateia | 4.02±0.15$^{d}$ | 2.44±0.08$^{d}$ | 39.30±0.17$^{c}$ |
| DK315M sterile | 2.70±0.05$^{f}$ | 2.36±0.08$^{e}$ | 12.59±0.09$^{f}$ |
| DK239MV | 3.79±0.10$^{d}$ | 2.32±0.01$^{e}$ | 38.79±0.10$^{c}$ |
| DN Meotyda | 5.47±0.23$^{c}$ | 2.14±0.01$^{f}$ | 60.88±0.23$^{a}$ |
| Kros256S | 6.63±0.23$^{b}$ | 3.71±0.03$^{a}$ | 44.04±0.23$^{b}$ |
| DK2831SV | 2.74±0.01$^{f}$ | 2.36±0.02$^{e}$ | 13.87±0.02$^{f}$ |
| Average in the hybrid group | 4.76±0.52* | 2.38±0.18** | 50.00±0.55*** |
| Average in the group of paternal forms | 4.63±0.44* | 2.55±0.11** | 44.92±0.45**** |

*Note*. Different letters within a column denote relative genotype groups formed based on the average values of the indicator and the margin of error. When comparing groups, averages with the same number of asterisks are not significantly different at the 0.05 significance level.

rotene content was recorded in the Kros268S sample (7.14±0.10 mg/kg), and the lowest in DK315M sterile (2.70±0.05 mg/kg). In addition to Kros268S, high (>6 mg/kg) initial β-carotene content was observed in DK3172 ZM (6.46 mg/kg), DB Tyras (6.48 mg/kg), and Kros256S (6.63 mg/kg).

After nine months of storage, the β-carotene content decreased in all maize genotypes, but the extent of the losses varied. The average β-carotene content decreased by 46.68% to 2.49 mg/kg of grain; however, no statistically significant differences by this parameter were found between the hybrids and their parental forms (2.38±0.18 and 2.55±0.11 mg/kg, respectively). The highest β-carotene content after storage was observed in Kros256S (3.71±0.03 mg/kg of grain), and the lowest — in DN Kyiakhy (1.98± ±0.03 mg/kg of grain).

The studied maize genotypes were conditionally divided into four groups based on the degree of the decrease in β-carotene content after storage: Group 1 — down to 20.0%, Group 2 — from 20.1% to 40.0%, Group 3 — from 40.1% to 60.0%, Group 4 — 60.1% and above. The first group included two inbreds, DK315M (sterile) and DK2831SV, which were characterized by a decrease in β-carotene content of less than 14% (12.59% and 13.87%, respectively). The β-carotene content at the time of grain harvesting and after storage for the DK315M inbred was 2.70 and 2.36 mg/kg, respectively, and for the DK2831SV inbred — 2.74 and 2.36 mg/kg. The second group comprised six genotypes with a 30–40% reduction in β-carotene content (DN Kyiakhy, Kros201S, DK7400SVZM, DK7400SVZM(2), DK239MV, and DN Halateia). These genotypes were characterized by β-carotene content ranging from 2.83 to 4.02 mg/kg at harvesting and from 1.98 to 2.46 mg/kg after storage. The third group included seven genotypes for which the decrease in β-carotene content was 44–55% (Kros256S, Kros160S, DK5002M, DN Sarmat, DB Tyras, DN Lehenda, and DK633/325MV). The β-carotene content for this group of genotypes ranged from 4.35 to 6.63 mg/kg at harvesting and from 2.03 to 3.71 mg/kg after storage. The fourth group included three genotypes in which β-carotene content losses during storage exceeded 60% (DK3172 ZM, DN Meotyda, and Kros268S), while the β-carotene content at the time of grain harvesting was 5.47–7.14 mg/kg and 2.14–2.70 mg/kg after storage. The results showed that β-carotene losses during storage are not always directly related to its absolute content after storage but are genotype-specific. Thus, the DK315M sterile inbred and the DK2831SV inbred were characterized by the lowest losses of β-carotene content (12.59% and 13.87%, respectively); however, after storage, the carotenoid content in their grain was only 2.36 mg/kg. In contrast, the Kros256S genotype, despite a significant reduction in β-carotene content (44.04%), had the highest β-carotene content after nine months of storage (3.71 mg/kg). Therefore, the absolute β-carotene content in the grain after storage is a more informative indicator for breeding aimed at increased post-storage provitamin A content, whereas β-carotene losses should be considered as an additional characteristic of the stability of the «β-carotene content» trait.

The two-way ANOVA analysis **(Table 2)** revealed a significant effect of genotype and duration of storage, as well as their interaction, on β-carotene content in maize grain. The effect of genotype accounted for 30.7%, storage duration for 51.7%, and the interaction of these two factors for 15.2%. Thus, although storage duration was the leading factor in the decrease in β-carotene content in this study, genotypic differences also played a significant role.

Among the investigated maize genotypes, polymorphism of carotenogenesis genes $lyc_{\varepsilon}$ and *crtRB1* was defined using the corresponding functional markers $lyc_{\varepsilon}$-SNP216 and *crtRB1*-3´TE **(Table 3, Fig. 1)**. Based on the $lyc_{\varepsilon}$-SNP216 marker, two allelic variants of the $lyc_{\varepsilon}$ gene were identified: the null-allele, corresponding to the presence of a nucleotide with the nitrogenous base, guanine (G) at the marker site (fa-

**Table 2.** The two-way ANOVA analysis of β-carotene content in maize grain samples (genotype × storage duration)

| Variance source | *df* | Sum of squares | Mean square | $F_{actual}$ | P | $F_{0.05}$ | $\eta^2x\pm\Delta$ |
|---|---|---|---|---|---|---|---|
| Genotype | 17 | 76.087 | 4.476 | 52.72 | 1.02E-33 | 1.77 | 30.7±7.2 |
| Storage duration | 1 | 128.315 | 128.315 | 1511.50 | 4.56E-50 | 3.97 | 51.7±0.7 |
| Genotype × storage duration | 17 | 37.638 | 2.214 | 26.08 | 4.33E-24 | 1.77 | 15.2±3.6 |
| Occasional deviations | 72 | 6.112 | 0.085 | — | — | — | 2.5 |
| Total | 107 | 248.152 | 135.089 | — | — | — | — |

**Table 3.** The allelic state of carotenogenesis gene markers in maize genotypes (bp)

| Genotype | Carotenogenesis markers | |
|---|---|---|
| | *lyc*$_{\varepsilon}$-SNP216 | *crtRB1*-3′TE |
| DN Kyiakhy | 395 (T) | 296 |
| Kros201S | 395 (T) | 296 |
| DK7400SVZM | 395 (T) | 296 |
| DN Sarmat | null-allele (G) | 296+875 |
| DK5002M | null-allele (G) | 296+875 |
| DK633/325MV | null-allele (G) | 296+875 |
| DB Tyras | 395 (T) | 296+875 |
| Kros160S | 395 (T) | 296+875 |
| DK7400SVZM(2) | 395 (T) | 296 |
| DN Lehenda | null-allele (G) | 296 |
| Kros268S | null-allele (G) | 296 |
| DK3172 ZM | null-allele (G) | 296 |
| DN Halateia | null-allele (G) | 296 |
| DK315M sterile | null-allele (G) | 296 |
| DK239MV | null-allele (G) | 296 |
| DN Meotyda | null-allele (G) | 296 |
| Kros256S | null-allele (G) +395 (T) | 296+543 |
| DK2831SV | null-allele (G) | 296 |

*Note*. There are two possible alleles for the *lyc*$_{\varepsilon}$-SNP216 marker: the null-allele, corresponding to the presence of a nucleotide with the nitrogenous base, guanine (G), at the marker site (favorable allele), and 395 bp, corresponding to the presence of a nucleotide with the nitrogenous base, thymine (T), at the marker site (unfavorable allele). For the *crtRB1*-3′TE marker, three alleles are possible: 543 bp (favorable), 296 bp, and 296+875 bp (unfavorable).

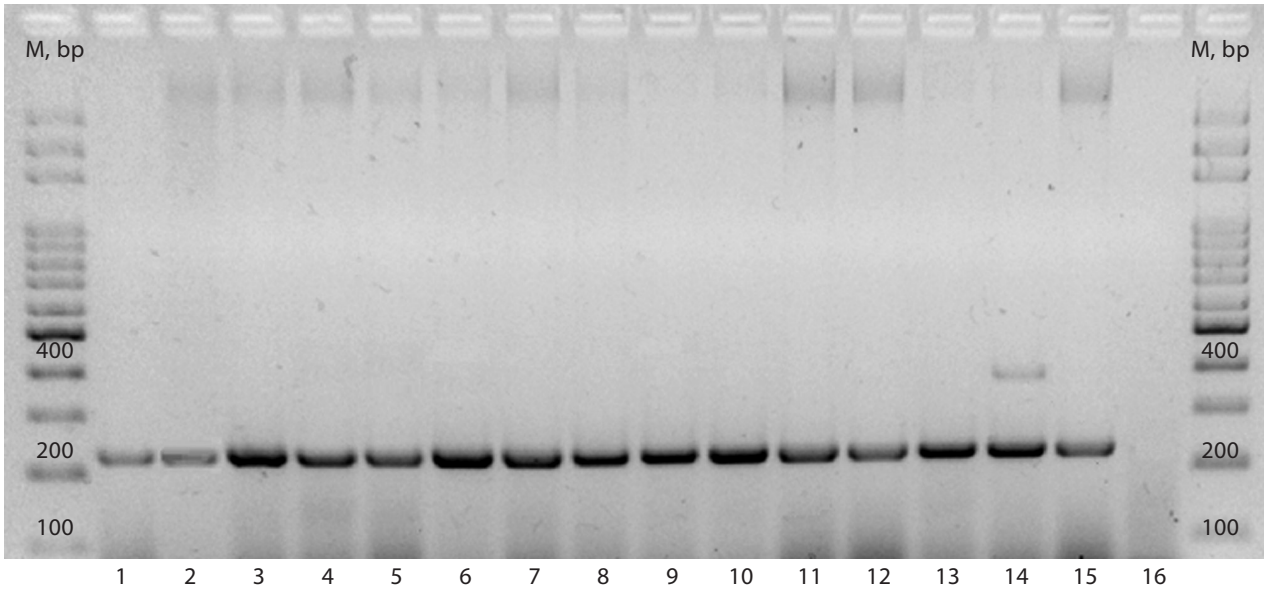


**Fig. 1.** The electrophoregram of amplification products with the *lyc*$_{\varepsilon}$-SNP216+*adh1* primer: M — molecular weight marker; 1–3 — DN Lehenda; 4 — Kros268S; 5 — DK3172 ZM; 6–8 — DN Halateia; 9 — DK315M sterile; 10 — DK239MV; 11–13 — Meotyda DNA; 14 — Kros256S; 15 — DK2831SV; 16 — no DNA (control)

vorable allele), and the 395 bp variant, corresponding to the presence of a nucleotide with the nitrogenous base, thymine (T) at this site (unfavorable allele). Based on the *crtRB1*-3´TE marker, three allelic variants of the *crtRB1* gene were identified: 543 bp (favorable), 296 bp, and 296+875 bp (unfavorable).

In most investigated combinations, the allelic composition of the hybrids corresponded to the genotypes of their parental forms, which was consistent with the expected pattern for the inheritance of carotenogenesis gene markers. At the same time, certain features in the allelic composition were noted for the DN Meotyda hybrid. In particular, in this hybrid, the electropherogram of amplification products using the $lyc_{\varepsilon}$-SNP216 primer to the $lyc_{\varepsilon}$ gene demonstrated a null-allele in a homozygous state, indicating that it was inherited from both parental components — the DK2831SV inbred **(Fig. 1)** and the Kros256S hybrid. At the same time, the 395 bp allele was visualized in Kros256S. Given its hybrid origin, it was assumed that this genotype was heterozygous: along with the detected 395 bp allele, it also contained the null-allele, which is not visualized on the electropherogram.

The favorable G allele of the $lyc_{\varepsilon}$ gene, as determined by the $lyc_{\varepsilon}$-SNP216 marker, was observed in eleven (61.1%) of the eighteen genotypes, while the unfavorable T allele was observed in six genotypes (33.3%). One (5.6%) genotype was heterozygous and contained both (G+T) alleles.

The favorable allele 543 bp of the *crtRB1* gene, as determined by the *crtRB1*-3´TE marker, was detected in one (5.6%) genotype — the single-cross hybrid Kros256S — in a heterozygous state. The other samples (94.4%) were characterized by unfavorable alleles — 296 bp (12 samples, 66.6%) and 296+ +875 bp (5 samples, 27.8%).

A comparison of maize genotype groups with different allelic states of the $lyc_{\varepsilon}$ gene based on the $lyc_{\varepsilon}$-SNP216 marker revealed certain differences in β-carotene content at the time of grain harvesting **(Table 4)**.

Genotypes with the G allele (null-allele) had an average of this indicator at 4.79±0.43 mg/kg, which, although not significantly different from the group with the T allele (395 bp) — 4.13 ± 0.55 mg/kg — it was accompanied by an upward trend compared to the T-group genotypes. In the heterozygous genotype (T+G) Kros256S, this indicator was significantly higher (6.63 ± 0.23 mg/kg) **(Table 1)** compared to the averages for the G and T groups, though it did not exceed the indicators for individual high-carotene genotypes within specified groups.

In the T genotype group, the median (3.61 mg/kg) was lower than the average, indicating a predominance of samples with low β-carotene content. In contrast, in the G group, the median (5.03 mg/kg) exceeded not only the average within its own group but also the median of the T genotype group, indicating generally higher β-carotene content among most genotypes in this group. The genotype groups with the G and T alleles were characterized by a similar level of variability in β-carotene content, as evidenced by similar coefficients of variation (29.7% and 33.0%, respectively), although the range of values in the G-allele carrier group was slightly wider (4.44 vs. 3.65 mg/kg).

Thus, the favorable G allele of the $lyc_{\varepsilon}$ gene, as determined by the $lyc_{\varepsilon}$-SNP216 marker, was associated with β-carotene content in maize grain at harvesting ranging from 2.70 to 7.14 mg/kg, with the average of 4.79 mg/kg. The unfavorable T allele was associated with β-carotene content ranging from 2.83 to 6.48 mg/kg, with the average of 4.13 mg/kg.

**Table 4.** Characterization of groups of maize genotypes with different allelic state of gene $lyc_{\varepsilon}$ (marker $lyc_{\varepsilon}$-SNP216) by the β-carotene content at time of harvesting

| Indicator | | Group of genotypes with T (395 bp) | Group of genotypes with G (null-allele) |
|---|---|---|---|
| Number of analyzed inbreds | | 6 | 11 |
| β-carotene content at the time of harvesting, mg/kg | average | 4.13±0.55[a] | 4.79±0.43[a] |
| | min-max | 2.83–6.48 | 2.70–7.14 |
| | range | 3.65 | 4.44 |
| | median | 3.61 | 5.03 |
| | V, % | 33.0 | 29.7 |

*Note*. When two groups are compared, the averages with the same letters are unreliably different at the significance level of 0.05.

A comparative analysis of maize genotype groups with different allelic states of the *crtRB1* gene (based on the *crtRB1*-3´TE marker) revealed differences in β-carotene content after nine months of storage and in the rate of its decrease **(Table 5)**.

A heterozygous genotype combining an unfavorable (296 bp) and a favorable (543 bp) allele of the *crtRB1* gene, as determined by the *crtRB1*-3´TE Kros256S marker, was characterized by a 1.5-fold higher average β-carotene content after nine months of storage (3.71±0.03 mg/kg) than the corresponding value (2.48±0.12 mg/kg) in the combined group of genotypes with the unfavorable alleles 296 and 296+875 bp. The value of this indicator in the genotype with the 296+543 bp allele significantly exceeded the maximum levels notable of the genotypes in the 296 bp and 296+875 bp groups.

At the same time, statistically significant differences were found in the average post-storage β-carotene content between the groups with the unfavorable alleles 296 bp and 296+875 bp: the first group contained 2.31±0.06 mg/kg, and the second — 2.65± ±0.10 mg/kg. The medians in the groups with unfavorable alleles practically coincided with the averages. The coefficient of variation for the studied groups with unfavorable alleles did not exceed 11%, indicating the homogeneity and stability of the distribution of β-carotene content values within each group.

As for the degree of the decrease in β-carotene content in maize grain after nine months of storage, no differences were observed among the studied genotype groups: in the heterozygous genotype (296+ +543 bp), the post-storage decrease in β-carotene content was 44.04%, and in the group with unfavorable alleles, it was 45.48%. The lack of differences in the degree of β-carotene content decrease among the studied groups is likely due to the limited presence of the 543 bp allele, which was found only in one genotype in a heterozygous state. This prevented a proper assessment of its effect on carotenoid degradation. To study the effect of *crtRB1* gene alleles on β-carotene content, it is advisable to expand the range of investigated genotypes, particularly those containing the favorable 543 bp allele of this gene.

At the same time, certain differences in post-storage β-carotene content were observed among groups of genotypes with unfavorable alleles. In the group with the 296 bp allele, the average decrease in β-carotene content was 39.42%, with a wide range of variation in values (12.59–62.18%), a high coefficient of variation (43.07%), and a relatively low median (37.14%). The group of genotypes with the 296+875 bp allelic state was characterized by greater decrease of β-carotene content (51.54%) compared to the group with 296 bp, a relatively narrow range of variation (49.50–54.11%), a low coefficient of

**Table 5.** Characterization of groups of maize genotypes with different allelic state of gene *crtRB1* (marker *crtRB1*-3´TE) by the β-carotene content after nine months of storage

| Indicator | | Group of genotypes with unfavorable alleles of gene *crtRB1* | | | Group of genotypes with a favorable allele of gene *crtRB1* in heterozygous state (296+543 bp) |
|---|---|---|---|---|---|
| | | 296 bp | 296+875 bp | 296 and 296+875 bp together | |
| Number of analyzed inbreds | | 12 | 5 | 17 | 1 |
| β-carotene content after 9 months of storage, mg/kg | Average | 2.31±0.06[a] | 2.65±0.10[b] | 2.48±0.12[ab] | 3.71±0.03[c] |
| | min-max | 1.98–2.57 | 2.51–3.04 | 1.98–3.04 | — |
| | range | 0.59 | 0.53 | 1.06 | — |
| | median | 2.34 | 2.57 | 2.44 | 3.71 |
| | V, % | 9.14 | 8.28 | 10.77 | — |
| Decrease in β-carotene content, % | average | 39.42±4.8[a] | 51.54±0.90[b] | 45.48±4.88[a] | 44.04±0.23[a] |
| | min-max | 12.59–62.18 | 49.50–54.11 | 12.59–62.18 | — |
| | range | 49.59 | 4.61 | 49.59 | — |
| | median | 37.14 | 51.30 | 49.50 | 44.04 |
| | V, % | 43.07 | 3.95 | 35.40 | — |

*Note*. When two groups are compared, the averages with the same letters are unreliably different at the significance level of 0.05.

variation (3.95%), and a median close to the average (51.30%). Thus, genotypes carrying the 296+875 bp allele proved to be more stable but were characterized by higher β-carotene content decrease during storage, whereas genotypes with the 296 bp allele demonstrated greater variability but, overall, lower carotenoid losses.

Thus, the unfavorable alleles of the *crtRB1* gene, as determined by the *crtRB1*-3´TE marker (296 bp, 296+875 bp), were associated with β-carotene content in maize grain after nine months of storage at the levels of 1.98–3.04 mg/kg, which corresponded to a decrease of 12.59–62.18% from the initial level. The limited availability of genotypes with the favorable allele of this gene (543 bp) did not allow us to investigate its effect on β-carotene losses during storage.

## DISCUSSION

The β-carotene content in maize grain is characterized by considerable variability and is influenced by a complex set of factors, including genotypic characteristics. The significant contribution of genetic factors to the formation of this trait is evidenced by substantial differences in β-carotene content among genotypes of maize inbreds of different origins: ranging from 5 to 50 mg/g of dry matter (A-R. Sayadi Maazou et al., 2022), from 1.5 to 12.4 mg/kg (A. Menkir et al., 2025), and from 0.38 to 1.78 mg/kg (K. Kljak et al., 2024).

Our results also indicate significant genotypic variability in this trait among grain samples of Ukrainian breeding. We found that the β-carotene content in the grain of the varieties studied at the time of harvesting varied within a wider range — from 2.70 to 7.14 mg/kg of grain, while after nine months of storage, the variation was smaller — from 1.98 to 3.71 mg/kg.

As shown by our results **(Table 2)**, the duration of maize grain storage had a more significant effect than that of genotype on the decrease in β-carotene content. While the average β-carotene content at the time of maize harvesting was 4.67 mg/kg of grain, after nine months of storage, this value decreased by 47%, to 2.49 mg/kg of grain. It is well known that carotenoids are susceptible to storage conditions, including temperature, moisture content, and the degree of grain grinding. Thus, β-carotene is better preserved in whole grains than in ground grains, due to less exposure to oxygen and light (Gunjević et al., 2024; Saenz et al., 2025). The presence of genotype-specific differences in the rate of β-carotene degradation during storage, even under identical grain storage conditions in our experiment, indicates genetically determined mechanisms of its stability. This is confirmed by the significant variability in β-carotene losses among the investigated genotypes, ranging from 13 to 62%. At the same time, the results showed that low β-carotene losses were not always accompanied by high β-carotene content after storage **(Table 1)**. Therefore, when assessing the breeding value of genotypes, it is advisable to consider not the stability of the «β-carotene content» trait, but the absolute β-carotene content in the grain after storage, since this indicator determines the potential nutritional value of the grain under conditions of long-term storage. From this perspective, genotypes that combine a high initial β-carotene content with a sufficiently high level after storage are of particular interest, notably Kros256S, Kros268S, and DK3172 ZM.

The genotypic influence on β-carotene content in maize grain is determined, in particular, by the allelic state of the carotenogenesis genes, among which the lycopene-ε-cyclase and β-carotene hydroxylase genes play a key role. The former determines the direction of the carotenoid metabolic pathway, while the latter controls the hydroxylation of β-carotene and, consequently, the extent of its retention. According to A-R. Sayadi Maazou et al. (2022), who investigated associations between the alleles of carotenogenesis genes and β-carotene content in 64 tropical maize inbreds, only a trend toward association with β-carotene content was observed for the $lyc_{\varepsilon}$-SNP216 marker, whereas for the *crtRB1*-3′TE marker, a highly significant association was established ($P < 0.0001$): inbreds with the favorable allele were characterized by higher content of this carotenoid. The lack of a significant association for the $lyc_{\varepsilon}$ gene is likely related to its role in directing the metabolic pathway of carotenoids during earlier stages of biosynthesis, whereas β-carotene content was assessed at later stages, when degradation processes play a significant role. Under these conditions, the contribution of the *crtRB1* gene becomes decisive.

A relatively weak association between the allelic state of the lycopene-ε-cyclase gene (as indicated by the *lcyε-5′*TE marker) and β-carotene content was also observed in 26 tropical maize genotypes of various genetic origins (Babu et al., 2013) and 10 inbreds of American breeding (R. Vallabhaneni et al., 2009). In particular, the increase in β-carotene content in maize genotypes with the favorable $lyc_{\varepsilon}$ allele was relatively

low, within the range of 0–30%, which was attributed to the complex regulation of carotenoid biosynthesis involving several genes (Babu et al., 2013).

Our results are consistent with these data and also indicate a weak association between the allelic state of the lycopene-ε-cyclase gene and β-carotene content in maize grain: the average β-carotene content was 4.79±0.43 mg/kg for genotypes with the favorable allele, compared to 4.13±0.55 mg/kg for genotypes with the unfavorable allele.

The importance of the *crtRB1* gene in controlling the β-carotene accumulation has been confirmed in the publication by other authors. For instance, H.S. Chauhan et al. (2025), via introgression of a favorable allele, obtained hybrids with a 7.8-fold increase in provitamin A content (19.52 vs. 3.33 mg/kg). Similar results are reported in studies by B.K. Mehta et al. (2024) and F. Hossain (2023), where the increase in β-carotene content was 5.2 and 5.7 times respectively. According to the data of I. Băcilă et al. (2022), inbred inbreds with a favorable allele of the *crtRB1* gene showed the highest β-carotene content, and inbreds with favorable alleles for both genes ($lyc_{\varepsilon}$ and *crtRB1*) had 60% higher β-carotene content than inbreds with two unfavorable alleles.

At the same time, the limited number of genotypes in our sampling characterized by a favorable allelic state of the *crtRB1* gene did not allow (at this stage) for a comprehensive analysis of the relationship between the alleles of this gene and β-carotene content. This highlights the need for further research aimed at expanding the genetic diversity of the source material and conducting an in-depth analysis of gene interactions in the regulation of carotenogenesis.

From a practical standpoint, genotypes that combine a higher β-carotene content in grain with a favorable allelic state of the carotenogenesis genes are of particular interest. Among the investigated samples, such genotypes include Kros268S and DK3172 ZM, which were characterized by high β-carotene content at the time of grain harvesting (7.14 and 6.46 mg/kg, respectively) and carried the favorable G allele of the $lyc_{\varepsilon}$ gene. The genotype Kros256S deserves special mention, as it had an elevated β-carotene content at harvesting (6.63 mg/kg) and the highest content after nine months of storage (3.71 mg/kg), and carried the favorable allele of 543 bp of the *crtRB1* gene in a heterozygous state, as well as the favorable allele G of the $lyc_{\varepsilon}$ gene in a heterozygous state. Based on the results obtained, the genotypes Kros256S, Kros268S, and DK3172 ZM can be considered as promising donors for breeding maize with a higher provitamin A content.

## CONCLUSIONS

The variability in β-carotene content in the grain was observed among maize genotypes of Ukrainian breeding both at harvesting and after nine months of storage: β-carotene content ranged from 2.70 to 7.14 mg/kg at harvesting and from 1.98 to 3.71 mg/kg after nine months of storage. It was found that β-carotene content in grain was determined by both genotypic characteristics and the duration of grain storage. The effect of genotype on β-carotene content in the grain was 30.7%, that of storage duration was 51.7%, and the combined effect of these two factors was 15.2%. Based on the $lyc_{\varepsilon}$-SNP216 marker of the lycopene-ε-cyclase gene, the favorable G allele was identified in 61.1% of the investigated genotypes, the unfavorable T allele — in 33.3%, and one heterozygous G/T genotype (5.6%); whereas for the *crtRB1*-3′TE marker, the unfavorable alleles of 296 and 296+875 bp predominated (94.4%). Genotypes carrying the favorable G allele of the $lyc_{\varepsilon}$ gene were characterized by a tendency toward higher β-carotene content in the grain compared to carriers of the T allele. The favorable G allele of the $lyc_{\varepsilon}$ gene, as determined by the $lyc_{\varepsilon}$-SNP216 marker, was associated with β-carotene content at harvesting ranging from 2.70 to 7.14 mg/kg, with an average of 4.79 mg/kg, whereas the T allele was associated with a range of 2.83–6.48 mg/kg, with an average of 4.13 mg/kg. At the same time, the limited number of genotypes with the favorable allele of 543 bp of the *crtRB1* gene did not allow for a comprehensive statistical analysis of its effect on β-carotene content, which highlights the need to expand the range of investigated genotypes, particularly those containing the favorable 543 bp allele of this gene. The most promising potential donors for maize breeding for increased provitamin A content were identified as the genotypes Kros268S and DK3172 ZM, which combined high β-carotene content in the grain with the favorable G allele of the $lyc_{\varepsilon}$ gene, as well as Kros256S, which was characterized by high β-carotene content both at harvesting and after storage, possessed the G allele of the $lyc_{\varepsilon}$ gene, and was the only one to carry the favorable 543 bp allele of the *crtRB1* gene in a heterozygous state. The results obtained can be used for selection of starting material and marker-assisted selection of maize for increased β-carotene content in grain.

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## REFERENCES

Babu R, Rojas NP, Gao S, et al (2013) Validation of the effects of molecular marker polymorphisms in *LcyE* and *CrtRB1* on provitamin A concentrations for 26 tropical maize populations. *Theor Appl Genet 126*(2):389–99. https://doi.org/10.1007/s00122-012-1987-3.

Băcilă I, Haş V, Ňuteu D, et al (2022) Screening of the Romanian maize (*Zea mays* L.) germplasm for *crtRB1* and lcyE alleles enhancing the provitamin A concentration in endosperm. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca 50*(3):12621. https://doi.org/10.15835/nbha50312621.

Bhatt V, Muthusamy V, Panda KK, et al (2023) Expression dynamics of lpa1 gene and accumulation pattern of phytate in maize genotypes possessing opaque2 and *crtRB1* genes at different stages of kernel development. *Plants 12*(9):1745. https://doi.org/10.3390/plants12091745.

Chandrasekharan N, Ramanathan N, Pukalenthy B, et al (2022) Development of β-carotene, lysine, and tryptophan-rich maize (*Zea mays*) inbreds through marker-assisted gene pyramiding. *Sci Rep 12*:8551. https://doi.org/10.1038/s41598-022-11585-y.

Chauhan HS, Zunjare RU, Rashmi T, et al (2025) Enrichment of vitamin A and vitamin E in sweet corn kernels through genomics-assisted introgression of mutant version of *crtRB1* and *vte4* genes. *Appl Biochem Biotechnol 197*(3):1889–905. https://doi.org/10.1007/s12010-024-05104-0.

Denysiuk KV, Satarova TM, Semenova VV, et al (2024) SNP analysis of Ukrainian maize inbreds with alternative state of molecular carotenogenesis marker *crtRB1-3*' TE. *Agricultural Science and Practice 11*(1):77–85. https://doi.org/10.15407/agrisp11.01.077.

Ewens WJ, Brumberg K (2023) Introductory statistics for data analysis. *Springer Cham*, p. 273. https://doi.org/10.1007/978-3-031-28189-1.

Gunjević V, Musa MM, Zurak D, et al (2024) Carotenoid degradation rate in milled grain of dent maize hybrids and its relationship with the grain physicochemical properties. *Food Research International 177*:113909. https://doi.org/10.1016/j.foodres.2023.113909.

Harjes CE, Rocheford TR, Bai L, et al (2008) Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. *Science 319*(5861):330–3. https://doi.org/10.1126/science.1150255.

Hossain F, Jaiswal SK, Muthusamy V, et al (2023) Enhancement of nutritional quality in maize kernel through marker-assisted breeding for *vte4*, *crtRB1*, and *opaque2* genes. *J Appl Genet 64*(3):431–43. https://doi.org/10.1007/s13353-023-00768-6.

Kljak K, Zurak D, Svečnjak Z, Grbeša D (2024) Relationship of physical properties and macronutrient composition with carotenoid profile in maize hybrids. *Agriculture 14*(3):384. https://doi.org/10.3390/agriculture14030384.

Mehta BK, Chauhan HS, Basu S, et al (2024) Mutant *crtRB1* gene negates the unfavourable effects of *opaque2* gene on germination and seed vigour among shrunken2-based biofortified sweet corn genotypes. *Funct Plant Biol 51*:FP23179. https://doi.org/10.1071/FP23179.

Menkir A, Dieng I, Meseka S, et al (2025) Concurrent enhancement of provitamin A and yield in tropical maize hybrids. *Front Plant Sci 16*:1611495. https://doi.org/10.3389/fpls.2025.1611495.

Mng'ong'o ME, Msungu SD (2024) Golden harvest: Nitrogen's impact on maize carotenoids. *Food and Humanity 3*:100371. https://doi.org/10.1016/j.foohum.2024.100371.

Munkhuwa V, Masamba K, Kasapila W (2023) Beta-carotene retention and consumer acceptability of selected products made from two provitamin-A maize varieties. *Int J Food Sci 2023*:5575291. https://doi.org/10.1155/2023/5575291.

Muthusamy V, Hossain F, Thirunavukkarasu N, et al (2015) Development of β-carotene rich maize hybrids through marker-assisted introgression of β-carotene hydroxylase allele. *PLoS One 10*(3):e0122130. https://doi.org/10.1371/journal.pone.0122130.

Rodriguez-Amaya DB, Kimura M (2004) Handbook for carotenoid analysis (2 ed.). HarvestPlus, Washington, pp 30–7.

Saenz E, Borrás L, Gerde JA (2025) Differential impact of storage temperature and endosperm hardness on carotenoid retention in maize whole grain and flour. *Journal of Cereal Science 126*:104294. https://doi.org/10.1016/j.jcs.2025.104294.

Sagare DB, Shetti P, Reddy SS, et al (2015) Identification of β-carotene rich maize inbreds using PCR-based assay for *crtRB1*-3'TE allele. *IJSN 6*(3):441–3.

Sayadi Maazou A-R, Gedil M, Adetimirin VO, et al (2021) Comparative assessment of effectiveness of alternative genotyping assays for characterizing carotenoids accumulation in tropical maize inbred lines. *Agronomy 11*(10):2022. https://doi.org/10.3390/agronomy11102022.

Šimić D, Galić V, Jambrović A, et al (2023) Genetic variability in carotenoid contents in a panel of genebank accessions of temperate maize from Southeast Europe.

*Plants 12*(19):3453. https://doi.org/10.3390/plants12193453.

Vallabhaneni R, Gallagher CE, Licciardello N, et al (2009) Metabolite sorting of a germplasm collection reveals the hydroxylase3 locus as a new target for maize provitamin A biofortification. *Plant Physiol 151*:1635–45. https://doi.org/10.1104/pp.109.145177.

Yan J, Kandianis CB, Harjes CE, et al (2010) Rare genetic variation at *Zea mays crtRB1* increases beta-carotene in maize grain. *Nat Genet 42*(4):322–7. https://doi.org/10.1038/ng.551.

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## ВМІСТ β-КАРОТИНУ В ЗЕРНІ ТА АЛЕЛЬНИЙ СТАН ГЕНІВ ЛІКОПІН-ε-ЦИКЛАЗИ ТА β-КАРОТИНГІДРОКСИЛАЗИ 1 У ГЕНОТИПІВ КУКУРУДЗИ УКРАЇНСЬКОЇ СЕЛЕКЦІЇ

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**Мета.** Виявити серед генотипів кукурудзи української селекції потенційні донори підвищеного вмісту β-каротину в зерні та сприятливих алелів генів лікопін-ε-циклази (*lyc*$_\varepsilon$) і β-каротингідроксилази 1 (*crtRB1*) шляхом оцінки вмісту β-каротину в зерні та аналізу асоціацій між вмістом β-каротину й алельним станом генів каротиногенезу. **Методи.** Спектрофотометричний аналіз, метод полімеразної ланцюгової реакції, статистичні методи. Вміст загальних каротиноїдів за β-каротином у зерні кукурудзи аналізували двічі: на момент збирання врожаю (вересень 2024 року) і через дев'ять місяців зберігання (червень 2025 року). **Результати.** Встановлено значну варіабельність вмісту β-каротину серед досліджених генотипів. На момент збирання зерна цей показник становив у середньому 4,67 мг/кг (2,70–7,14 мг/кг), а після дев'яти місяців зберігання знижувався в середньому на 46,68% і становив 2,49 мг/кг (1,98–3,71 мг/кг). Ступінь втрат істотно варіювала залежно від генотипу (12,59–62,18%). Двохфакторний дисперсійний аналіз показав, що найбільший внесок у варіацію ознаки «вміст β-каротину» в зерні мала тривалість зберігання (51,7%), тоді як частка генотипу становила 30,7%, а їх взаємодія — 15,2%. За маркером *lyc*$_\varepsilon$-SNP216 гена лікопін-ε-циклази ідентифіковано генотипи з алелем G (61,1%), алелем T (33,3%) та один гетерозиготний генотип G/T (5,6%), тоді як за маркером *crtRB1*-3′TE гена β-каротингідроксилази 1 переважали несприятливі (296 і 296+875 п.н.) алелі (94,4%). Виявлено тенденцію до підвищення вмісту β-каротину у генотипів зі сприятливим алелем G гена *lcyε* (4,79±0,43 мг/кг) порівняно з генотипами з T-алелем (4,13±0,55 мг/кг). Генотип Крос256С із гетерозиготним станом *crtRB1* (296+543 п.н.) характеризувався підвищеним післязберігальним вмістом β-каротину (3,71 мг/кг). В той же час наявність хоча й обмеженої кількості генотипів зі сприятливим (543 п.н.) алелем, надає можливість в наступному провести повноцінний статистичний аналіз щодо впливу сприятливих алелів цього гена на післязберігальний вміст β-каротину. З'ясовано, що найбільш перспективними потенційними донорами для селекції кукурудзи на підвищений вміст провітаміну А є генотипи Крос268С і ДК3172 ЗМ, які поєднували високий вміст β-каротину із наявністю сприятливого алеля G гена *lyc*$_\varepsilon$, а також Крос256С, який характеризувався високим вмістом β-каротину як на момент збирання зерна, так і після дев'яти місяців зберігання та містив сприятливі алель G гена *lyc*$_\varepsilon$ і алель 543 п.н. гена *crtRB1*. **Висновки.** Для генотипів кукурудзи української селекції комплексно оцінено вміст β-каротину в зерні на момент збирання та після дев'яти місяців зберігання у поєднанні з визначенням алельного стану генів *lcy*$_\varepsilon$ і *crtRB1*. Встановлено особливості прояву алельного стану генів *lcy*$_\varepsilon$ і *crtRB1* щодо варіабельності вмісту β-каротину в зерні та його збереження. Серед генотипів кукурудзи української селекції виявлено потенційні донори підвищеного вмісту β-каротину в зерні та сприятливих алелів генів каротиногенезу *lyc*$_\varepsilon$ і *crtRB1*. Оцінено генотипову варіабельність вмісту β-каротину в зерні на момент збирання зерна і після дев'яти місяців зберігання. Генотипи-носії сприятливого алеля G гена *lyc*$_\varepsilon$ характеризувалися тенденцією до підвищеного вмісту β-каротину в зерні, тоді як оцінка впливу сприятливого алеля 543 п.н. гена *crtRB1* потребує розширення вибірки досліджуваних генотипів. Генотипи Крос268С, ДК3172 ЗМ і Крос256С можуть розглядатися як найбільш перспективні потенційні донори для селекції кукурудзи на підвищений вміст β-каротину в зерні. Отримані результати можуть бути використані для добору вихідного матеріалу та маркер-асоційованої селекції кукурудзи на підвищений вміст β-каротину в зерні.

**Ключові слова:** *Zea mays* L., lcyε, *crtRB1*, молекулярно-генетичні маркери, каротиноїди, генетична варіабельність, поліморфізм ДНК, провітамін А, зберігання зерна.