

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(7): 1054-1057
www.biochemjournal.com
Received: 12-04-2026
Accepted: 18-05-2026

Sandesh Goragundi Mahesh
Department of Plant Breeding
and Genetics, Agricultural
College and Research Institute,
Madurai, Tamil Nadu
Agricultural University,
Coimbatore, Tamil Nadu,
India

Bharathi Pukalenty
Department of Plant Breeding
and Genetics, Agricultural
College and Research Institute,
Madurai, Tamil Nadu
Agricultural University,
Coimbatore, Tamil Nadu,
India

Nandini B
Department of Genetics and
Plant Breeding, University of
Agricultural Sciences,
Dharwad, Karnataka, India

Nagalakshmi Ramanathan
Centre for Plant Breeding and
Genetics, Tamil Nadu
Agricultural University,
Coimbatore, Tamil Nadu,
India

Senthil Natesan
Centre for Plant Molecular
Biology and Biotechnology,
TNAU, Coimbatore, Tamil
Nadu, India

Corresponding Author:
Sandesh Goragundi Mahesh
Department of Plant Breeding
and Genetics, Agricultural
College and Research Institute,
Madurai, Tamil Nadu
Agricultural University,
Coimbatore, Tamil Nadu,
India

Post-harvest evaluation of high β -carotene maize (*Zea mays* L.) derived through marker-assisted introgression

Sandesh Goragundi Mahesh, Bharathi Pukalenty, Nandini B, Nagalakshmi Ramanathan and Senthil Natesan

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i7Sm.9177>

Abstract

Vitamin A deficiency (VAD) is a critical global health issue, especially in regions where maize is a staple. While standard maize contains low levels of provitamin A (PVA) carotenoids, this study utilized Marker-Assisted Breeding (MAB) to introgress the favorable *crtRB1* allele into 11 adapted elite inbred lines using two high beta-carotene Universal Maize Inbred (UMI) testers. Molecular confirmation verified the successful transfer of the target 543 bp allele in all 22 F1 hybrids. The hybrid N 14 $\times$ UMI 1200-7-26-1-6-1 was identified as a superior genotype, accumulating 6.27 μ g/g of beta-carotene. Post-harvest evaluations demonstrated that roti preparation offered the highest beta-carotene retention (67.87%), while *in vitro* digestion models revealed a high bio-accessibility recovery rate of 69.51%. These findings confirm that biofortified maize is a viable and effective strategy for delivering essential micronutrients to combat VAD.

Keywords: Biofortified, *Zea mays*, bioavailability and marker-assisted breeding

Introduction

Maize (*Zea mays* L.) is a vital global cereal, providing up to 61% of caloric intake in some developing nations, yet typical varieties are severely deficient in PVA carotenoids, containing only 0.15 to 1.5 μ g/g. This deficiency is a primary driver of childhood blindness and increased mortality rates globally. The carotenoid biosynthetic pathway in maize is regulated by the *crtRB1* gene, which encodes β -carotene hydroxylase 1; the favorable allele 1 (543 bp) is known to reduce the conversion of β -carotene into non-PVA compounds, thereby increasing its accumulation in the endosperm (Yan *et al.*, 2010 and Vinodhana *et al.*, 2025)^[13, 11]. For biofortification to be effective, these crops must not only possess high PVA content but also maintain nutritional stability during post-harvest processing (Garrett *et al.*, 1999; Burt *et al.*, 2010; De Oliveira and Rodriguez-Amaya, 2007; Ekpa, *et al.*, 2021 and Zurak *et al.*, 2025)^[4, 1, 2, 3, 14] and show high bioavailability for human absorption.

Materials and Methods

Genetic Material and Hybridization: Eleven elite inbred lines (females) were crossed with two high beta-carotene UMI testers in a Line $\times$ Tester design at the Agricultural Research Station, Bhavanisagar.

Marker-Assisted Screening: Foreground selection for the *crtRB1* 3'TE gene was conducted using specific primers (65F, 62R, 66R) to identify the favourable 543 bp allele.

Extraction and HPLC Quantification of Beta-Carotene

Seeds harvested from the hybrids and their corresponding parental lines were evaluated for β -carotene content. To minimize carotenoid degradation, seeds were harvested at approximately 14% moisture content and stored at 22-26 °C until analysis. β -Carotene extraction and quantification were performed according to the HarvestPlus protocol described by Rodriguez *et al.* (2004)^[9], with appropriate precautions to prevent photo-oxidative losses.

Seed samples were finely ground in ice-cold acetone, and all subsequent extraction procedures were conducted under yellow light (Quackenbush, 1963; Weber, 1987) [8, 12]. The carotenoid extracts were concentrated under reduced pressure using a rotary evaporator at 45 °C and reconstituted in methanol to a final volume of 2 mL. β -Carotene was separated and quantified using high-performance liquid chromatography (HPLC) equipped with a C18G 120A column (250 $\times$ 4.6 mm) and a photodiode array detector set at 450 nm. The mobile phase consisted of acetonitrile, methanol, and ethyl acetate (80:10:10, v/v/v) and was delivered at a flow rate of 1 mL min⁻¹. A calibration curve was generated using an authentic β -carotene standard (M/s. Sigma Aldrich, India) prepared in methanol at concentrations of 0.1, 1, 10, 50, and 100 μ g/g β -Carotene was identified by matching its characteristic absorption spectrum and chromatographic retention time with those of the authentic standard. Quantification was subsequently performed using the corresponding standard calibration curve.

Bioavailability and Post-Processing Retention

Provitamin A bio-accessibility was determined *via* an *in vitro* stimulated gastrointestinal digestion model according to the procedure given by Garrett (1999) [4] and Lakshminarayana *et al.*, (2009) [5] with slight modification in both beta carotene introgressed and normal inbred lines. All analyses were performed in triplicate for raw maize samples, *in vitro* digested samples, and processed food products. Digestive stability (bio-availability) was determined by calculating the ratio of β -carotene recovered after the oral and intestinal phases of *in vitro* digestion to the initial β -carotene content present in the untreated maize samples. Post-processing retention was evaluated by cooking biofortified raw maize flour into traditional food products: *popcorn*, *chapati*, *roti*, and *noodles*, tracking terminal beta-carotene survival.

Data analysis was carried out using STATA statistical software (StataCorp version 14.2). Descriptive statistics, including the mean and standard deviation (SD), were calculated from triplicate measurements to evaluate β -carotenoid retention in processed maize samples. The effects of processing on β -carotene levels were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test. Statistical significance was defined as $p < 0.05$.

Results and Discussion

Beta-Carotene Accumulation, Bio-accessibility and Post-Processing Retention

The nutritional enhancement objective was conclusively met. HPLC quantification revealed that standard elite lines possessed negligible beta-carotene *i.e.*, N 42 at 0.07 μ g/g and N 104 at 0.10 μ g/g, representing severe baseline deficiency (Table 1). The donors UMI 1200-7-26-1-6-1 and UMI 1230-2-70-9-6-1 naturally accumulated 8.40 μ g/g and

6.44 μ g/g, respectively. Estimation of β -carotene in hybrids revealed that among the 22 hybrids, the hybrid N 14 $\times$ UMI 1200-7-26-1-6-1 showed high β -carotene content followed by the hybrids N 5 $\times$ UMI 1200-7-26-1-6-1, N 90 $\times$ UMI 1200-7-26-1-6-1 and N 14 $\times$ UMI 1230-2-70-9-6-1. The top-performing agronomic hybrid N 14 $\times$ UMI 1200-7-26-1-6-1 synergistically accumulated a high beta-carotene content of 6.27 μ g/g, making it the paramount dual-purpose cross.

In vitro-stimulated gastrointestinal digestion of the biofortified hybrid cross (UMI 1200 β $\times$ UMI 1230 β) showed an initial beta-carotene content of 7.41 μ g/g, which decreased to 5.15 μ g/g post-digestion (Table 2). This represents a remarkably high bio accessibility recovery rate of 69.51%, significantly superior to that of standard, non-fortified maize (49.38%) (Figure 1).

Because carotenoids are structurally sensitive to isomerization and oxidation through heat and light, post-processing retention was evaluated. Thermal food processing of biofortified raw flour initially (6.38 μ g/g) led to varying degrees of degradation depending on the cooking method. In case of processed food products as expected the raw maize flour has a carotenoid content of (6.38 $\pm$ 0.171 μ g/g) which was higher when compared to the concentration (0.25-2.5 μ g/g) generally reported for typical yellow maize varieties (Nuss and Tanumihardjo, 2010) [6] but still lower than the current breeding target for biofortified maize as set by HarvestPlus (17 μ g/g of Provitamin A). The retention of β -carotene in the individual processed products *roti*, *chapati* and the *noodles* and *popcorns* revealed that, the exposure of β -carotene for high temperature and for prolonged time will reduce the retention of β -carotene and among these food products popcorn retained 57.68% (3.68 μ g/g), *chapati* displayed the absolute lowest retention at just 27.90% (1.78 μ g/g) and in *noodles* a moderate retention of 45.77% (2.92 μ g/g) was observed (Table 3), since these were considerably exposed to light and temperature for long time when compared to *roti* which exhibited the highest retention at 67.87% (4.33 μ g/g). As supported by Ortiz-Monasterio *et al.* (2007), β -carotene is affected by light, storage, and other environmental factors. These results indicate that additional research is required to improve the food processing methods to increase the retention of carotenoid concentration in maize hybrids, to reach the target set by HarvestPlus. This, in turn, reduces PVA deficiency and malnutrition.

Crucially, the bio accessibility and postharvest fate of beta-carotene determine the real-world success of this intervention. The structural integrity of carotenoids renders them deeply susceptible to thermal breakdown (Sowa *et al.*, 2017) [10]. The processing data clearly show that severe dry heat (*e.g.*, *chapati*, *popcorn*) destroys the vast majority of provitamin A. However, the preparation of *roti* has nearly 68% retention and a 63.47% digestive bio-accessibility, indicating that biofortified maize provides a highly effective, metabolizable dietary matrix for delivering provitamin A directly to vulnerable demographics.

Table 1: Beta carotene content in parents and hybrids

Sl. No	Genotypes	β -carotene content ($\mu\text{g/g}$)
1	UMI 1200-7-26-1-6-1	8.40
2	UMI 1230-2-70-9-6-1	6.44
3	N5	0.25
4	N14	1.58
5	N 27	0.12
6	N 42	0.07
7	N 66	0.33
8	N 90	0.79
9	N 94	1.07
10	N 104	0.10
11	N 107	1.35
12	N 117	0.59
13	N 285	1.69
14	N 5 $\times$ UMI 1200-7-26-1-6-1	5.66
15	N 14 $\times$ UMI 1200-7-26-1-6-1	6.27
16	N 27 $\times$ UMI 1200-7-26-1-6-1	3.74
17	N 42 $\times$ UMI 1200-7-26-1-6-1	4.85
18	N 66 $\times$ UMI 1200-7-26-1-6-1	5.06
19	N 90 $\times$ UMI 1200-7-26-1-6-1	5.62
20	N 94 $\times$ UMI 1200-7-26-1-6-1	2.66
21	N 104 $\times$ UMI 1200-7-26-1-6-1	5.31
22	N 107 $\times$ UMI 1200-7-26-1-6-1	2.20
23	N 117 $\times$ UMI 1200-7-26-1-6-1	3.22
24	N 285 $\times$ UMI 1200-7-26-1-6-1	2.91
25	N 5 $\times$ UMI 1230-2-70-9-6-1	2.98
26	N 14 $\times$ UMI 1230-2-70-9-6-1	5.48
27	N 27 $\times$ UMI 1230-2-70-9-6-1	3.43
28	N 42 $\times$ UMI 1230-2-70-9-6-1	2.75
29	N 66 $\times$ UMI 1230-2-70-9-6-1	2.44
30	N 90 $\times$ UMI 1230-2-70-9-6-1	1.85
31	N 94 $\times$ UMI 1230-2-70-9-6-1	4.54
32	N 104 $\times$ UMI 1230-2-70-9-6-1	1.45
33	N 107 $\times$ UMI 1230-2-70-9-6-1	4.42
34	N 117 $\times$ UMI 1230-2-70-9-6-1	1.58
35	N 285 $\times$ UMI 1230-2-70-9-6-1	2.77

Table 2: β -carotene content in raw maize flour samples and invitro digested samples.

S. No	Samples	β -carotene ($\mu\text{g/g}$)	
		Raw Maize flour Mean $\pm$ SD	<i>In vitro</i> accessibility Mean $\pm$ SD
1	UMI 1200	2.39 $\pm$ 0.020*	1.18 $\pm$ 0.033*
2	UMI 1200 β	6.35 $\pm$ 0.016*	4.03 $\pm$ 0.292*
3	UMI 1200 β $\times$ UMI 1230 β	7.41 $\pm$ 0.040*	5.15 $\pm$ 0.220*

Value of means $\pm$ SD, each analysis was carried out in triplicate. *significant at $p < 0.05\%$

Table 3: β -carotene content in raw maize flour and the processed food products and their percentage of retention:

S. No	Food Samples	β -carotene ($\mu\text{g/g}$) Mean $\pm$ SD	Retention (%)
1	Raw maize flour	6.38 $\pm$ 0.171 *	100.00
2	Popcorn	3.68 $\pm$ 0.776 *	57.68
3	Chapati	1.78 $\pm$ 0.369 *	27.90
4	Roti	4.33 $\pm$ 0.177 *	67.87
5	Noodles	2.92 $\pm$ 0.094 *	45.77

Mean $\pm$ SD, *statistically significant at $p < 0.05$

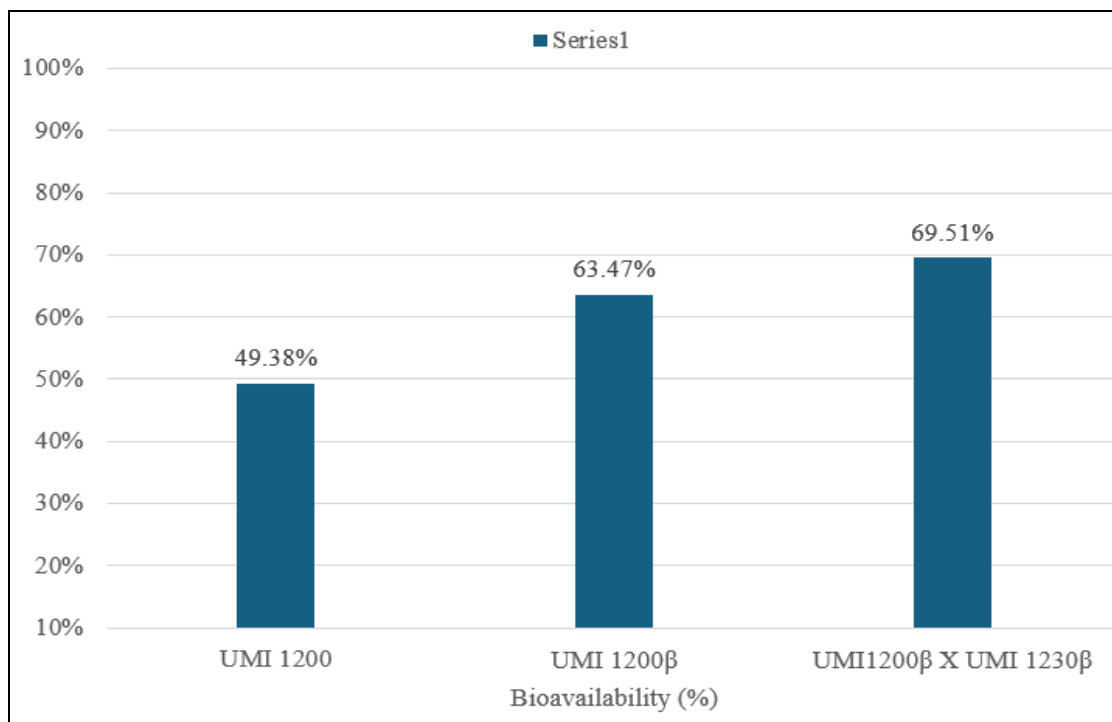


Fig 1: Comparison of β -carotene bioavailability between normal maize samples and *in vitro* digested samples.

Conclusion

The study concludes that integrating MAB for the *crtRB1* allele is a highly efficient method for developing elite biofortified maize hybrids. The hybrid N 14 $\times$ UMI 1200-7-26-1-6-1 was identified as the paramount genotype, combining high yield potential with a beta-carotene content of 6.27 μ g/g. Its superior digestive bio-accessibility (69.51%) and high retention during roti preparation demonstrate that biofortified maize is a ready-to-deploy tool to systematically alleviate Vitamin A deficiency and "hidden hunger" globally.

References

- Burt AJ, Grainger CM, Young JC, Shelp BJ, Lee EA. Impact of postharvest handling on carotenoid concentration and composition in high-carotenoid maize (*Zea mays* L.) kernels. *J Agric Food Chem*. 2010;58(14):8286-8292.
- De Oliveira GP, Rodriguez-Amaya DB. Processed and prepared corn products as sources of lutein and zeaxanthin: Compositional variation in the food chain. *J Food Sci*. 2007;72(1):S079-S085.
- Ekpa O, Fogliano V, Linnemann A. Carotenoid stability and aroma retention during the post-harvest storage of biofortified maize. *J Sci Food Agric*. 2021;101(10):4042-4049.
- Garrett DA, Failla ML, Sarama RJ. Development of an *in vitro* digestion method to assess carotenoid bioavailability from meals. *J Agric Food Chem*. 1999;47(10):4301-4309.
- Lakshminarayana R, Raju M, Keshava Prakash MN, Baskaran V. Phospholipid, oleic acid micelles and dietary olive oil influence the lutein absorption and activity of antioxidant enzymes in rats. *Lipids*. 2009;44(9):799-806.
- Nuss ET, Tanumihardjo SA. Maize: A paramount staple crop in the context of global nutrition. *Compr Rev Food Sci Food Saf*. 2010;9(4):417-436.
- Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Pena RJ. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *J Cereal Sci*. 2007;46(3):293-307.
- Quackenbush FW. Corn carotenoids: Effects of temperature and moisture on losses during storage. *Cereal Chem*. 1963;40:266-269.
- Rodriguez-Amaya DB, Kimura M. *HarvestPlus Handbook for Carotenoid Analysis*. Washington (DC): International Food Policy Research Institute; 2004.
- Sowa M, Yu J, Palacios-Rojas N, Goltz SR, Howe JA, Davis CR, *et al*. Retention of carotenoids in biofortified maize flour and β -cryptoxanthin-enhanced eggs after household cooking. *ACS Omega*. 2017;2(10):7320-7328.
- Vinodhana NK, Ravikesavan R, Senthil N, Sivakumar S, Datta HM, Mohanapriya B. Enrichment of β -carotene content in maize inbred UMI 1201 through marker assisted back cross breeding. *Electron J Plant Breed*. 2025;16(3):323-330.
- Weber EJ. Carotenoids and tocopherols of corn grain determined by HPLC. *J Am Oil Chem Soc*. 1987;64(8):1129-1134.
- Yan J, Kandianis CB, Harjes CE, Bai L, Kim EH, Yang X, *et al*. Rare genetic variation at *Zea mays crtRB1* increases β -carotene in maize grain. *Nat Genet*. 2010;42(4):322-327.
- Zurak D, Gunjević V, Svečnjak Z, Kiš G, Grbeša D, Kljak K. Carotenoid stability in grains of maize hybrids compared to complete feeds for laying hens following preparation and storage. *Appl Food Res*. 2025;5(1):100855.