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Research Article

Enrichment of β carotene content in maize inbred UMI 1201 through marker assisted back cross breeding

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Abstract
Maize is a good source of provitamin A for humans. A promising approach to eliminating Vitamin A deficiency (VAD) in developing countries like India is to biofortify maize genotypes with micronutrients like β - carotene. This study was therefore designed to increase the β-carotene concentration in the maize inbred UMI 1201, the female parental line of the popular maize hybrid COH(M)8 by marker assisted backcross breeding (MABB). The *crtRB1* enriched β carotene inbred, UMI 1230β⁺, was utilized as the donor parent. In the MABB program, *crtRB1* 3'TE, the gene-specific marker for β carotene was used for foreground selection and 105 SSR markers were used for background selection. Four enhanced UMI 1201 lines for β carotene concentration were identified with good agronomic performance and recurrent parent genome recovery (RPGR) ranging from 91.0 to 94.0percent. The improved lines outperform the recurrent parent UMI 1201 in terms of β carotene accumulation which ranged from 7.73 to 10.37 µg/g., with UMI 1201β⁺-2 being the most effective. The grain yield of the improved lines ranged from 2308 kg/ha to 2519 kg/ha. Morphological resemblance of the improved lines recorded more than 90% of phenotypic similarity with the recurrent parent UMI 1201 for the traits *viz.,* anthocyanin colouration of the brace roots (present), attitude of leaf blade (straight), leaf width of blade (Narrow), tassel anthocyanin colouration of glume excluding the base (present), tassel anthocyanin of anthers (present), ear anthocyanin coloration of silks (present), ear shape (cylindrical) and kernel row arrangement (straight). The introgressed inbred possessing the *crtRB1* allele can be used as donor for β carotene enrichment in the biofortification program.

Keywords: Maize, Biofortification, β-carotene, MABC

INTRODUCTION

Millions of people in developing countries relay on maize to meet their calorie requirement. The process of fortifying food with vital nutrients is a very successful way to combat micronutrient deficiencies in developing nations (Satyanarayana, 2024). Maize is one of the food vehicles used for fortification, as it serves as the staple food crop worldwide. It exhibits considerable diversity in the composition of grain carotenoid profiles with respect to the predominant carotenoids (lutein and zeaxanthin), provitamin A carotenoids (α-carotene, β-carotene and β-cryptoxanthin) and other non-provitamin A carotenoids (zeinoxanthin). Vitamin A, a micronutrient that is vital to human health, is derived from provitamin A carotenoids. People who are primarily dependent on cereals lacking in provitamin A are affected by vitamin A deficiency, which is very common throughout the world (Gedil *et al.,* 2024) Vitamin A deficiency deteriorates the immune system and raises the risk of infectious diseases such as diarrhea and measles, resulting in slowed growth and xeropthalmia, a principal source of blindness in children.In this context, maize has been considered as an important target crop under the Harvest plus challenge program for increasing

provitamin A levels the target level of β-carotene has been set as 15 µg/g to, reduce VAD (Gautam *et al*., 2021). Therefore, women and children could consume 200–400 g of maize per day to meet their needs. While commercial fortification of foods, dietary diversification and supplementation are some of the strategies that have been used to combat VAD. Biofortification employing Marker Assisted Backcross Breeding (MABB) appears to be a viable and sustainable strategy to develop provitamin A biofortified maize.

Transfer of the genes related to enhancement of proA, with recurrent parent genetic background through backcross breeding program is effective approach. Marker Assisted Backcrossing (MABC) is regarded as the simplest form of marker-assisted selection, using desired marker linked to the proA is most acceptable method to develop a high proA lines (Muthusamy *et al*., 2012). Using molecular markers could also help reduce the high cost of HPLC (High Performance Liquid Chromatography) analyses to estimate the kernel carotenoids among the individuals of segregating populations.

Marker-Assisted Back Cross Breeding (MABC) programs are extensively used for provitamin A biofortification in maize as DNA markers associated with valuable alleles of *crtRB1* and *lcyE* genes were identified, validated and utilized in MAS and introgression (Babu *et al*., 2013). The amount of β-carotene in the kernels of 105 maize inbreds with Indian and CIMMYT ancestry was investigated by Muthusamy *et al*. (2014) which revealed the kernel β-carotene concentrations ranged from 0.02 to 16.50µg/g. Therefore, it is necessary to comprehend the key genes in the carotenoid biosynthesis pathway that may be altered to raise the concentration of β-carotene. The β-carotene hydroxylase enzyme (*crtRB1*) on chromosome 10 and lycopene epsilon cyclase (*lcyE*) on chromosome 8 are the two primary genes associated with β-carotene accumulation in maize (Harjes *et al*., 2008; Yan *et al.,* 2010). Of these, the gene *crtRB1* was found to have a significantly stronger impact on β-carotene concentrations than *lcyE* where it increases the β-carotene concentration two to ten fold due to the positive allele, *crtRB1* 3'TE (allele 1, 543 bp) (Babu *et al*., 2013). Several studies showed that this allele-based marker could effectively detect the *crtRB1* allele in diverse maize genotypes (Muthusamy *et al*., 2014; Natesan *et al*., 2020; Zunjare *et al*., 2018).

In this study, attempt has been made to introgress the *crtRB1* allele in the female parental line UMI 1201 of the maize hybrid COH(M)8 currently serving as the national check in AICRP maize trials and is well-suited to India's various agro-climatic zones. The hybrid is resistant against several diseases viz., Charcoal Rot, *Turcicum* leaf blight and Downy mildew and is suitable for both rainfed and irrigated cultivation (Nallathambi *et al*., 2012). The improved version of released maize hybrids QPM 9, HM 4, HM 8 and HM 9 demonstrated the success of MABB in enhancing the nutritional characters (Hossain *et al*., 2018; Sagare *et al*., 2019).Natesan *et al*. (2020) improved the β-carotene concentration in the COH (M) 6 parental lines *viz*., UMI 1200 and UMI1230 by using marker-assisted backcross breeding (MABB) to transfer the β-carotene gene, *crtRB1,* using the maize genotype HP467-15 as the donor. The β-carotene concentration in the improved lines varied between 7.056 and 9.232µg/g. In light of the importance of provitamin A biofortification and the possibility that the *crtRB1* gene could increase the amount of β-carotene in maize kernels, the current study aimed to introduce the *crtRB1* allele using MABB into the female parental line UMI 1201 of the COH(M) 8 hybrid; and to evaluate the improved lines for β carotene concentration and agronomic performance.

MATERIALS AND METHODS

Plant Materials : The seed materials of the recurrent parent UMI 1201, the female parent of the maize hybrid COH(M) 8 was obtained from the Maize unit, Department of Millets and the donor parent UMI 1230 β^+ introgressed with *crtRB1* allele for the high β-carotene concentration (9.232 µg/g) was obtained from the Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore.

Marker Assisted Introgression of the Maize inbred UMI 1201 for enhanced β carotene : The conversion of maize inbred with high β carotene involves crossing of the recurrent parent UMI 1201 with the donor parent UMI 1230 β^+, backcrossing (two generations) and selfing (two generations). The field experiments were conducted from 2020–21 to 2024–25 at the Department of Millets, New Area Farm, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. A *crtRB1* gene-specific marker *(crtRB1* 3'TE) was used for foreground selection (Yan *et al*.,2010) to choose plants from selfed and backcrossed generations. Foreground selection was focused on heterozygous plants with alleles 1, 543 bp (favorable allele) and 3, 296 bp (unfavorable allele) or homozygous plants with allele 1, 543 bp (favorable allele). Background selection was used to select the foreground positive plants with the highest recurrent parent genome recovery (RPGR) for the backcross and selfed generations using polymorphic simple sequence repeat (SSR) markers

During *rabi* 2020-21, crossing was effected between the inbreds UMI 1201 and UMI 1230β^+ to obtain the F_1 seeds. In *kharif* 2021, the F_1 plants were screened for the heterozygosity and backcrossed with the recurrent parent UMI 1201 to develop BC_1F_1 off springs. In *rabi* 2021-22, BC_1F_1 plants were derived through crossing of recurrent parent and 90 plants were screened for heterozygosity and the selected plants with high recurrent parent genome recovery were back crossed with the recurrent parent to develop BC_2F_1 progenies. The 75 BC_2F_1 plants were further screened for the heterozygosity during *kharif* 2022

and the chosen plants were selfed in order to get BC_2F_2 progenies. During *rabi* 2022-23, the 154 BC_2F_2 progenies were evaluated for the presence of favorable allele 1 (543bp) and the positive plants were selfed to get BC_2F_3 plants. The heterozygous plants with unfavourable allele 3 (296bp) and the favourable allele of 1 (543bp) and the homozygotes with unfavourable allele 3, (296bp) were rejected and excluded. Agro-morphological character evaluation and β-carotene estimation were carried out in BC_2F_3 improved lines during *kharif* 2023.

Genomic DNA Isolation and Marker Genotyping :The cetyl trimethyl ammonium bromide (CTAB) method was used to separate the genomic DNA from the young maize leaves. The amount and quality of the DNA were assessed using 0.8% agarose gel. Using the *crtRB1* gene-specific marker (*crtRB1* 3′TE), foreground selection was carried out (65F: ACACCACATGGACAAGTTCG, 6 2 R : A C A C T C T G G C C C A T G A A C A C , 66R:ACAGCAATACAGGGGACCAG).Background selection was conducted using 105 maize specific SSR markers that were uniformly distributed throughout the maize genome. SSR primer sequences were obtained from Eurofins Ltd, Bangalore, India. which were sourced from maize genome database (www.dotmaizegdb). The PCR for *crtRB1* 3'TE/SSR markers and gel electrophoresis were performed in accordance with the protocols of Pukalenthy *et al*. (2019) and Muthusamy *et al*. (2014).

The goodness of fit for genotyping result was assessed using the chi square analysis. The amplicons of the markers were scored as “A” for the recurrent parent, “B” for the donor parent, and “H” for heterozygotes.

Evaluation of the improved lines for agro-morphological characters : Multi location evaluation of the improved lines were also carried out during *kharif* 2024 at Department of Millets, TNAU, Coimbatore, Maize Research Station, Vagarai and Agricultural Research Station, Bhavanisagar in irrigated condition to assess the agro-morphological and yield traits. The trials were laid out in a plot size of 5 × 3.6 m in a randomized block design replicated thrice with spacing of 60 × 25 cm . All the recommended agronomic practices were followed. The β carotene content and agro-morphological traits [plant height (cm), days to 50% tasseling (days), days to 50% silking (days), cob length (cm), cob diameter (cm) number of kernel rows/ cob, number of kernels/row, 100 kernel weight (g) and grain yield/ plant (g)] were recorded in five selected plants per genotype based on the IBPGR maize DUS descriptors (Anonymous, 1991). Analysis of variance was appropriately carried for the traits under study and the β carotene content (µg/g) across the improved lines, recurrent and donor parent was compared using the Least Significant Difference (LSD) test.

Estimation of β-carotene concentration using HPLC analysis : The improved lines of UMI 1201 *viz*., UMI 1201β^+ - 1, UMI 1201β^+ - 2, UMI 1201β^+ - 3 and UMI 1201β^+ - 4 and seeds from the donor (UMI 1230 β^+) and recurrent parents (UMI 1201) were evaluated for β-carotene concentration. Due to the light sensitivity of the carotenoid compounds, the seeds were harvested at a moisture content of 14 percent and stored between 22 and 26 degrees Celsius. β-carotene content in grains was estimated by following the Harvest plus protocol (Rodriguez and Kimura, 2004). The seed samples were ground into a fine powder using ice-cold acetone in order to extract the carotenoid compounds. Additional processing was carried out under yellow light because the carotenoid compounds undergo photo-oxidation (Weber, 1987). A rotary evaporator was used to concentrate the extracted samples at 45°C. Before separation, methanol was added to make up to 2 milliliters. To determine the amount of β-carotene, high-performance liquid chromatography (HPLC) was used. A C18G 120A column (250 × 4 point 6mm) eluted the samples, which were then measured using a photodiode array detector set at 450 nm. The mobile phase was made up of acetonitrile, methanol, and ethylacetate (80:10:10) and was circulated at a rate of 1 milliliter per minute at high pressure by the column.The β-carotene reconstituted to five distinct concentrations (0.1, 1.0; 10.0; 50.0 and 100 µg/g) in methanol was utilized to create the standard curve. Their distinctive spectra and retention durations in comparison to recognized standard solutions made it possible to determine the concentration of β-carotene

RESULTS AND DISCUSSION

Marker-assisted introgression of the *crtRB1* gene in to UMI 1201**:** The Marker Assisted Backcross Breeding (MABB) scheme was used to enrich the β-carotene in UMI 1201, the female parental line of COH(M) 8 as reported previously by Muthusamy *et al*. (2014) and Zunjare *et al*. (2018), where they generated the improved high β-carotene hybrids Vivek QPM-9, HQPM7 and HQPM5. Consistent with this conclusion, Natesan *et al*. (2020) developed six β carotene enriched lines with good agronomic performance and β-carotene concentration ranging from 7.056 to 9.232 µg/g. The recurrent parent genome recovery of the β carotene improved lines ranged from 90 to 92 %.

In the present investigation, foreground and background selection were used in conjunction with a recurrent backcrossing procedure to introgress the *crtRB1* allele into UMI 1201. Transferring the gene of interest in MABB with high precision is made possible by foreground selection utilizing gene-linked markers. The selection of foreground positive plants with a high RPGR was aided by marker-assisted background selection employing SSR markers (Hossain *et al*., 2018.) A set of 105 SSR markers was used to screen for parental polymorphisms between the donor and recurrent parents; 62 of these markers were found to be polymorphic. Background selection and RPGR analysis were conducted using these polymorphic SSR

markers. The *crtRB1* gene-specific marker (*crtRB1* 3'TE) used in this study detected the positive plants without any false positives at any stage of MABB.

In this study, the F_1 plants derived from the cross UMI 1201 X UMI 1230β$^+$ were confirmed for the heterozygosity with *crtRB1*3'TE marker. The F_1 plants were backcrossed to the recurrent parent UMI 1201 and the resulting BC_1F_1 population (90 plants) were screened with *crtRB1*3'TE marker. A total of 21 heterozygous plants (allele 1/allele 3) were identified. The BC_1F_1 16 plants exhibited a maximum RPGR of 76% and was further selected to produce the BC_2F_1 population. A total of 75 plants of BC_2F_1 population were screened with a *crtRB1*3' TE marker of which 18 heterozygous (allele1/allele3) plants were identified and the percentage of RPGR in 14 positive plants was about 80.2%. To create the BC_2F_2 population, the top five BC_2F_1 plants were further chosen and selfed. A total of 154 BC_2F_2 plants were screened with *crtRB1*3'TE marker to identify the homozygous plants with allele1 (**Fig. 2**). It revealed 22 homozygous plants (allele 1) and the percentage of RPGR of positive plants ranged from 88.0–94.0%. Based on the RPGR and phenotype data, the numbers of selected plants were further reduced to four and selfed to produce BC_2F_3 lines. Hence, four BC_2F_3 improved lines (UMI 1201β$^+$-1, UMI 1201β$^+$-2, UMI 1201β$^+$-3 and UMI 1201β$^+$-4) were developed which showed 91.0 – 94.0 % RPGR with an average of 92.25%.

In this study, the segregation pattern of alleles 1 and 3 deviated from the expected Mendelian ratio (1:1 and 1:2:1) and allele 1 was diminished (**Table 1**). These findings were consistent with Babu *et al*. (2013), who reported allele 1 displays segregation distortion in five of the eight populations.

Multi location evaluation of the improved lines for agro-morphological traits: The β carotene enriched improved lines were evaluated in multi-location to assess the agronomic performance and the β-carotene content at Department of Millets, TNAU, Coimbatore, Maize Research Station, Vagarai and ARS, Bhavanisagar along with the checks UMI 1201 (Recurrent parent) and UMI 1230 β$^+$ (**Table 2, Table 3**). Characterizing and selection of the progenies that are more similar to the recurrent parent in terms of both morphological and nutritional traits is crucial for verifying the suitability of the new genotypes, as this selection enhances the MABB program. Therefore, agro-morphological characteristics of the improved lines were thus examined. It was revealed that the improved lines recorded more than 90% of phenotypic similarity with the recurrent parent UMI 1201 for the morphological traits viz., anthocyanin colouration of the brace roots (present), attitude of leaf blade (straight), leaf width of blade (Narrow), tassel anthocyanin colouration of glume excluding the base (present), tassel anthocyanin of anthers (present), ear anthocyanin coloration of silks (present), ear shape

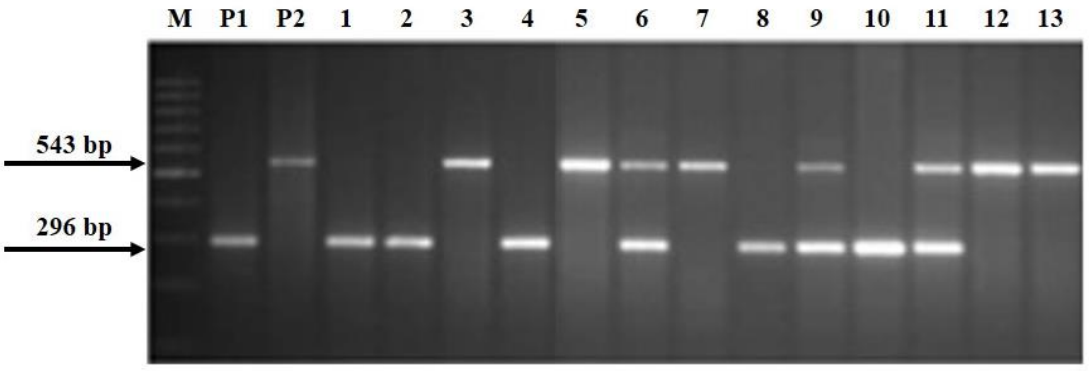

Fig 2. Segregation of allele1 and allele 3 in BC_2F_2 generation of UMI 1201 × UMI 1230β+ using the *crtRB1* gene specific marker (i.e., crtRB1 3'TE). **(M)** Ladder (100 bp), **(P1)** UMI1201, **(P2)** UMI 1230β+ and **(1–13)** BC_2F_2 progenies

Table 1. Segregation pattern of alleles of the *crtRB1* gene in back crossed and selfed progenies

Cross	Generation	Population size	No of homozygotes (allele1/allele1)	No of heterozygotes (allele 1/allele3)	No of homozygotes (allele3/allele3)	χ2	p-value
UMI 1200 × UMI 1230β$^+$	BC_1F_1	90	-	21	69	25.6	0.0001*
	BC_2F_1	75	-	18	57	20.3	0.0001*
	BC_2F_2	154	22	72	60	19.4	0.0005*

* Significant at P< 0.05

Table 2. β carotene concentration and agronomic performance of the improved lines developed through MABB in Multi location trials

Location	INBRED	Days to 50% tasseling	Days to 50% silking	Plant height (cm)	Cob length (cm)	Cob width (cm)	Number of kernel rows/ cob	Number of kernels/ row/cob	100 grain weight (g)	Grain yield (kg/ha)	β carotene (µg/g)
Coimbatore 2024-25 (*kharif*)	UMI 1201β⁺ - 1	54	57	155.5	13.8	5.0	12	30	28.0	2356	9.146
	UMI 1201β⁺ - 2	55	58	157	14.5	4.6	14	28	26.0	2545	10.338
	UMI 1201β⁺ - 3	54	56	159.4	15.0	5.2	12	29	26.5	2294	7.625
	UMI 1201β⁺ - 4	55	58	154.0	13.6	4.7	12	31	28.0	2470	7.423
	UMI 1201 **(RP)**	55	57	158.5	14.0	5.3	12	27	26.4	2125	0.885
	UMI 1230 β⁺ **(DP)**	54	57	162.3	15.5	5.0	14	28	28.0	2362	9.020

Location	INBRED	Days to 50% tasseling	Days to 50% silking	Plant height (cm)	Cob length (cm)	Cob width (cm)	Number of kernel rows/ cob	Number of kernels/ row/cob	100 grain weight (g)	Grain yield (kg/ha)	β carotene (µg/g)
Vagarai 2024-25 (*Rabi*)	UMI 1201 β⁺ -1	55	58	145.3	12.6	4.5	12	26	26.0	2286	9.076
	UMI 1201β⁺ - 2	56	60	151.7	13.7	4.2	12	29	29.0	2497	10.220
	UMI 1201β⁺ - 3	54	57	150.6	14.0	5.0	14	28	28.5	2315	8.625
	UMI 1201β⁺ - 4	55	58	148.0	12.0	4.0	14	30	30.6	2416	7.746
	UMI 1201 **(RP)**	54	57	152.5	13.6	4.5	12	27	27.4	2194	0.912
	UMI 1230 β⁺ **(DP)**	55	58	155.3	13.0	5.0	12	29	27.5	2358	9.124

Location	INBRED	Days to 50% tasseling	Days to 50% silking	Plant height (cm)	Cob length (cm)	Cob width (cm)	Number of kernel rows/ cob	Number of kernels/ row/cob	100 grain weight (g)	Grain yield (kg/ha)	β carotene (µg/g)
Bhavanisagar 2024-25 *kharif*	UMI 1201 β⁺ -1	54	56	152.6	14.7	4.7	12	27	26.5	2305	9.680
	UMI 1201β⁺ - 2	53	55	156.2	12.0	5.0	14	29	27.0	2514	10.560
	UMI 1201β⁺ - 3	55	58	157.0	13.7	4.5	12	27	28.2	2316	8.524
	UMI 1201β⁺ - 4	55	57	153.4	15.0	5.2	12	26	26.4	2396	8.025
	UMI 1201 **(RP)**	53	56	152.0	14.0	4.6	12	28	27.0	2150	1.025
	UMI 1230 β⁺ **(DP)**	56	58	160.5	15.0	4.5	14	29	28.0	2365	9.105

Table 3. Overall performance of the improved lines developed through MABB in Multi location trials

Entries	Days to 50% tasseling	Days to silking	Plant height (cm)	Cob length (cm)	Cob width (cm)	Number of kernel rows/cob	Number of kernels/row/ cob	100 grain weight (g)	Grain yield (kg/ha)	β carotene (µg/g)
UMI 1201β⁺ - 1	54	57	151.1	13.7	4.7	12	28	27.5	2316	9.30
UMI 1201β⁺ - 2	55	58	155.0	13.4	4.6	14	29	28.0	2519	10.37
UMI 1201β⁺ - 3	54	57	155.7	14.2	4.9	14	28	26.8	2308	8.25
UMI 1201β⁺ - 4	55	58	151.8	13.5	4.6	14	29	27.8	2427	7.73
SE	0.16	0.19	1.13	0.18	0.07	0.27	0.30	0.37	50.1	0.58
SD	0.32	0.38	2.26	0.37	0.13	0.54	0.61	0.74	100.2	1.17
UMI 1201 (RP)	54	57	154.3	13.9	4.8	12	27	26.2	2156	0.94
UMI 1230 β⁺ (DP)	55	58	159.4	14.5	4.8	14	29	27.4	2365	9.08

(cylindrical), kernel row arrangement (straight) (**Fig. 3**). The overall performance of the improved lines for the biometrical traits viz., days to 50% tasseling, days to 50% silking, plant height, cob length, cob diameter, number of kernels per row and 100 kernel weight showed that the traits recorded were onpar with their recurrent parent.

The grain yield of the improved lines ranged from 2308 kg/ha to 2519 kg/ha. Among the improved lines, UMI 1200β⁺-2 recorded maximum grain yield of 2519 kg/ha. These results were in accordance with the study of Zunjare *et al*. (2018), Chandran *et al*. (2019) and Shetti *et al*. (2020).

β-carotene concentration in improved lines: In this study, the β carotene content (µg/g) across the improved lines, recurrent and donor parents tested at different locations (Department of Millets, Coimbatore, ARS, Bhavanisagar and MRS, Vagarai) was compared using the Least Significant Difference (LSD) test, with UMI 1201 (RP) serving as the baseline (**Table 2, Table 3**.). The UMI 1201β⁺ - 2 introgressed line exhibited the highest β carotene content, with a mean value of 10.37 µg/g, significantly higher than the recurrent parent UMI 1201 (RP), which had a mean of 0.94 µg/g ($p < 0.05$). Similarly, the UMI 1201β⁺ - 1 line, with a mean of 9.30 µg/g, also showed a significant increase in β carotene content

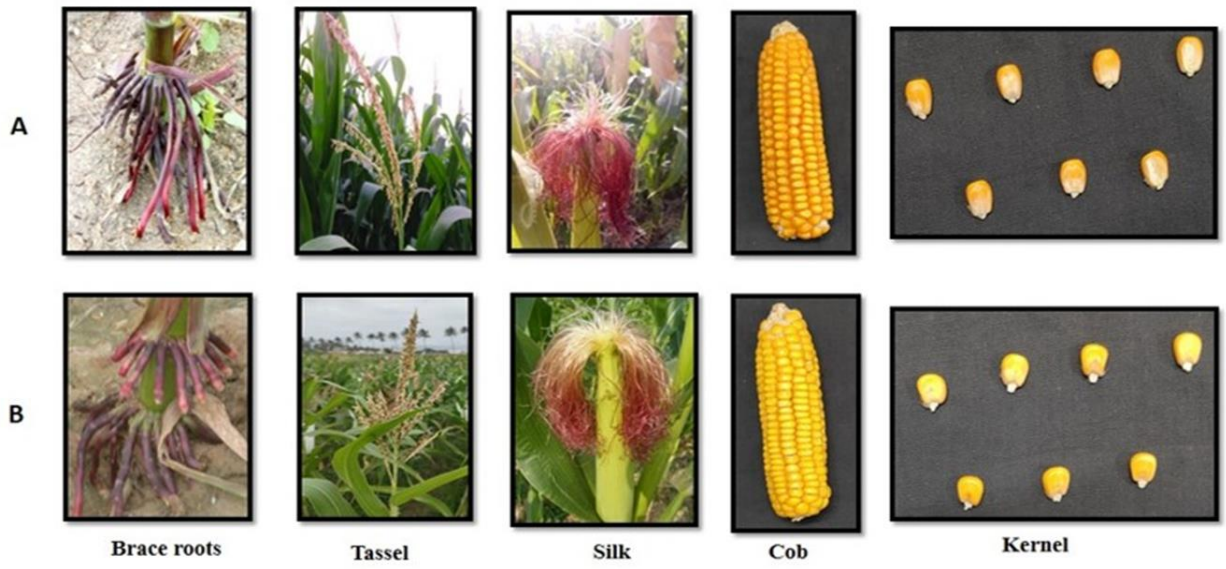

Fig 3. Morphological resemblance of the recurrent parent UMI 1201 (A) with the β Carotene improved line (B)

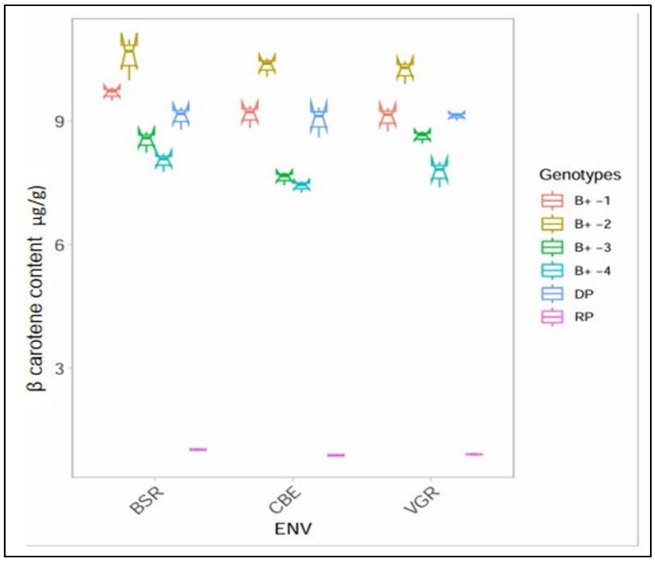

Fig. 4. β carotene content (µg/g) across the improved lines of UMI 1201, recurrent and donor parents tested at different locations

compared to the recurrent parent. The UMI 1201β⁺ - 3 line, with a mean of 8.26 µg/g, and UMI 1201β⁺ - 4 with 7.73 µg/g, also showed significantly higher β carotene content than the recurrent parent (0.941 µg/g) (**Fig. 4**).

These results suggest that the introgressed lines UMI 1201β⁺ - 1, UMI 1201β⁺ - 2, UMI 1201β⁺ - 3 and UMI 1201β⁺ - 4 significantly outperform the recurrent parent UMI 1201 (RP) in terms of β carotene accumulation, with UMI 1201β⁺-2 being the most effective. The enhanced lines can be regarded as potential parents for creating β-carotene hybrids that are suited to boost the quality improvement programme.

The only practical solution to address vitamin A deficiency is to biofortify maize with provitamin A carotenoids. The enriched lines of UMI 1201 in this study exhibited a significant 10-fold increase in kernel β-carotene, indicating that the introduction of allele 1 of the *crtRB1* gene alone significantly influences the accumulation of β-carotene at higher concentrations. The high recovery of RPG in the parental lines makes the improved lines to retain a similar grain yield potential. The current study, which reports on the rapid development of β carotene-enriched maize through the use of marker-assisted breeding, has great potential because it accurately selects desirable plants and does away with the need for extensive biochemical valuation in the segregating generations. The introgressed inbred UMI 1201 with the *crtRB1* allele can serve as a donor for β carotene enrichment in the biofortification program.

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