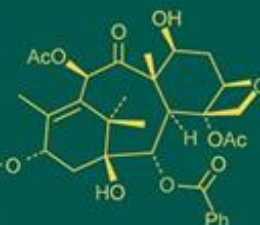
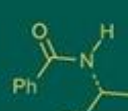
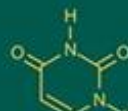
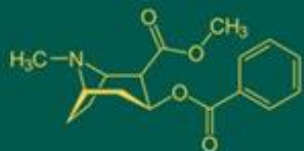


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(7): 1193-1202
www.biochemjournal.com
Received: 07-05-2026
Accepted: 09-06-2026

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

Bharathi Pukalenty
Department of Plant Breeding
and Genetics, Agricultural
College and Research Institute,
Madurai, 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, India

Development and validation of high β -Carotene Maize (*Zea mays* L.) lines through marker-assisted introgression of the *crtRB1* allele

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

DOI: <http://www.doi.org/10.33545/26174693.2026.v10.i7So.9202>

Abstract

Vitamin A deficiency (VAD) is a severe global malnutrition challenge, causing vision impairment and increasing susceptibility to diseases, particularly in populations relying on maize as a dietary staple. Typical yellow maize cultivars have low levels of provitamin A (PVA) carotenoids. This study aimed to develop biofortified, high beta-carotene maize hybrids utilizing Marker-Assisted Breeding (MAB) to introgress the favourable *crtRB1* allele into adapted inbred lines. Eleven adapted elite lines were crossed with two high beta-carotene Universal Maize Inbred testers in a Line $\times$ Tester mating design. Successful introgression was verified using a *crtRB1* 3'TE gene-specific marker, confirming heterozygosity (543 bp + 296 bp) in all 22 F₁ hybrids. Biometrical evaluation indicated a preponderance of non-additive gene action for most yield traits. The hybrid N 14 $\times$ UMI 1200-7-26-1-6-1 emerged as the superior cross, exhibiting standard heterosis of 26.73% and 51.01% over the commercial checks CO 6 and NK 6240 for grain yield (147.23 g/plant), along with a high beta-carotene content of 6.37 μ g/g. The yield advantage, along with biofortified lines, will help in combating malnutrition in the major population.

Keywords: *Zea mays* L, marker-assisted breeding, *crtRB1*, bio fortification, specific combining ability

1. Introduction

Maize (*Zea mays* L.) is a paramount cereal crop cultivated extensively worldwide, utilized for human consumption, livestock feed, and industrial raw materials. Possessing the highest production among all cereals, worldwide maize output reached 861 million tonnes in 2015-16, providing up to 61% of the caloric intake in various developing countries (Shiferaw *et al.*, 2013 and Satyanarayana, 2024) [25, 22]. While a typical maize kernel is calorie-dense, comprising approximately 73% starch and 9% protein commonly cultivated varieties suffer from a severe deficiency in provitamin A (PVA) carotenoids, typically accumulating a mere 0.15 to 1.5 μ g/g (Harjes *et al.*, 2008) [8]. This micronutrient shortfall significantly contributes to Vitamin A deficiency (VAD), a crisis that causes blindness in children and women, increases mortality rates globally, and severely impacts developing nations (Zunjure *et al.*, 2018) [36]. Unlike other major cereals, maize uniquely synthesizes coloured carotenoid compounds in its endosperm that the human body can metabolize into vitamin A (Bouis and Welch, 2010; Wurtzel, 2004) [4, 34]. In the carotenoid biosynthetic pathway, the *crtRB1* gene (encoding β -carotene hydroxylase 1) is critical; it converts β -carotene (which holds 100% provitamin A activity) into downstream, non-PVA compounds like β -cryptoxanthin and zeaxanthin (Babu *et al.*, 2013) [3]. Natural genetic variation in the *crtRB1* 3'TE region results in three allelic forms. Crucially, the favourable allele 1 (543 bp, without insertion) reduces transcript expression, stunting the hydroxylation of β -carotene and promoting its accumulation in the endosperm (Yan *et al.*, 2010 and Vinodhana *et al.*, 2025) [35, 32].

Biofortification which is, the enhancement of crop nutritional value through breeding offers a highly sustainable and cost-effective strategy to combat "hidden hunger" (Qaim *et al.*, 2007; Hotz and McClafferty, 2007) [19, 9]. Through Marker-Assisted Selection (MAS), breeders can bypass lengthy phenotypic evaluations by tracking target alleles directly, vastly accelerating the breeding cycle (Tanksley *et al.*, 1989; Warburton *et al.*, 2010 and Natesan *et al.*, 2020) [31, 33, 15]. However, for biofortified crops to be adopted by farmers, they must pair enhanced nutrition with superior agronomic performance.

This requires the thorough evaluation of General Combining Ability (GCA) and Specific Combining Ability (SCA) to harness heterosis, as yield is a highly complex quantitative trait (Sprague and Tatum, 1942) [29].

2. Materials and Methods

2.1. Plant genetic materials and hybridization

The breeding material comprised of 11 highly adapted elite inbred lines (N 5, N 14, N 27, N 42, N 66, N 90, N 94, N 104, N 107, N 117, and N 285) acting as female lines which were developed by Department of Biotechnology, Agricultural College and Research Institute, Madurai, and two high beta-carotene converted Universal Maize Inbreds (UMI 1200-7-26-1-6-1 and UMI 1230-2-70-9-6-1) acting as testers, which were developed at Department of Millets, Centre of Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Crossing was executed utilising the Line $\times$ Tester (L $\times$ T) mating design via the tassel bag method to generate 22 F₁ cross combinations. Parents, hybrids along with two standard checks CO 6 and NK 6240 were evaluated in a Randomized Block Design (RBD) with two replications. The experiment was carried out in Agricultural Research Station, Bhavanisagar, TamilNadu.

2.2. Marker-Assisted Screening (MAS) for the *crtRB1* Allele

Genomic DNA was isolated from 15-20 days old seedlings using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) extraction buffer protocol (Dellaporta, 1983) [5]. DNA quality was verified via 0.8% agarose gel electrophoresis. Polymerase Chain Reaction (PCR) amplification utilized *crtRB1* 3'TE gene-specific primers (65F: ACACCACATGG ACAAGTTTCG, 62R: ACACCTCTGGCCCATGAACAC, 66R: ACAGCAATACAGGGGACCAG) was carried out following the procedure of Muthusamy *et al.* (2014) and was used for foreground selection. SSR primer sequences were obtained from Eurofins Ltd, Bangalore. The amplified products were resolved on a 3% agarose gel to detect the favourable allele 1 (543 bp) and unfavourable allele 2 (296 bp).

2.3. Biometrical evaluation

Sixteen quantitative traits were recorded which includes days to 50% tasselling (days), Days to 50% silking (days), plant height (cm), Leaf length (cm), Leaf width (cm), Length to first cob (cm), number of branches per tassel, Tassel length (cm), cob length (cm), cob girth (cm), cob weigh (g), Number of kernel rows per ear, Number of kernels per row, 100-grain weight (g), single plant grain yield (g) and Shelling percentage (%) which were recorded according to the plant stage based on the standard maize descriptors formulated by IBPGR (Annon, 1991) [2].

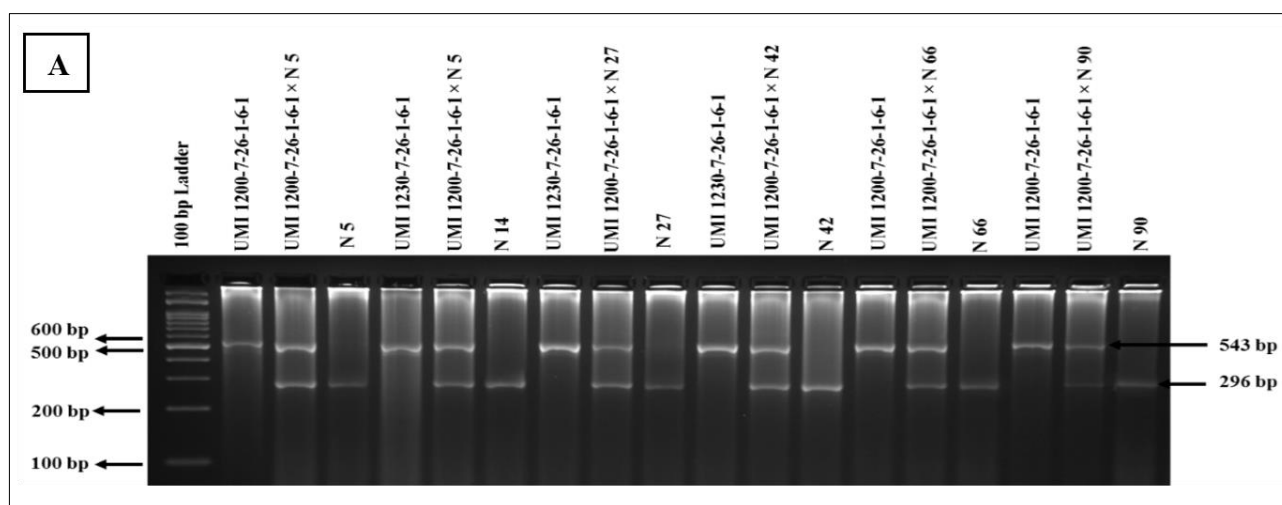
2.4 Statistical Analysis

Analysis of variance (ANOVA) (Panse and Sukhatme, 1964) [16], and combining ability parameters (GCA and SCA) were estimated. Standard heterosis was computed relative to checks. Genotypic correlation and path coefficient analyses (Dewey and Lu, 1959) were conducted to establish relationships between yield and morphological traits using the TNAU stat, software.

3. Rresults

3.1 Marker-Assisted Allelic introgression and Molecular Confirmation of F₁ Hybrids

Before taking up the hybridization PCR screening utilizing the *crtRB1* 3'TE specific markers definitively confirmed the genetic status of all experimental materials. The 11 elite inbred lines (N 5, N 14, N 27, N 42, N 66, N 90, N 94, N 104, N 107, N 117, and N 285) universally possessed the unfavourable wild-type allele (296 bp). Conversely, the two high beta-carotene Universal Maize testers UMI 1200-7-26-1-6-1 and UMI 1230-2-70-9-6-1 possessed the target favourable allele 1 (543 bp). The *crtRB1* allele was introgressed into 11 lines by crossing two high beta-carotene converted Maize Inbreds UMI 1200-7-26-1-6-1 and UMI 1230-2-70-9-6-1 acting as testers to generate 22 hybrids using Line $\times$ Tester design. Most crucially, all 22 F₁ cross combinations amplified both the 543 bp and 296 bp fragments. This dual-band profile confirmed true heterozygosity across the progeny, proving that the MAB protocol successfully and uniformly transferred the *crtRB1* trait into the diverse, elite genetic backgrounds (Figure 1).



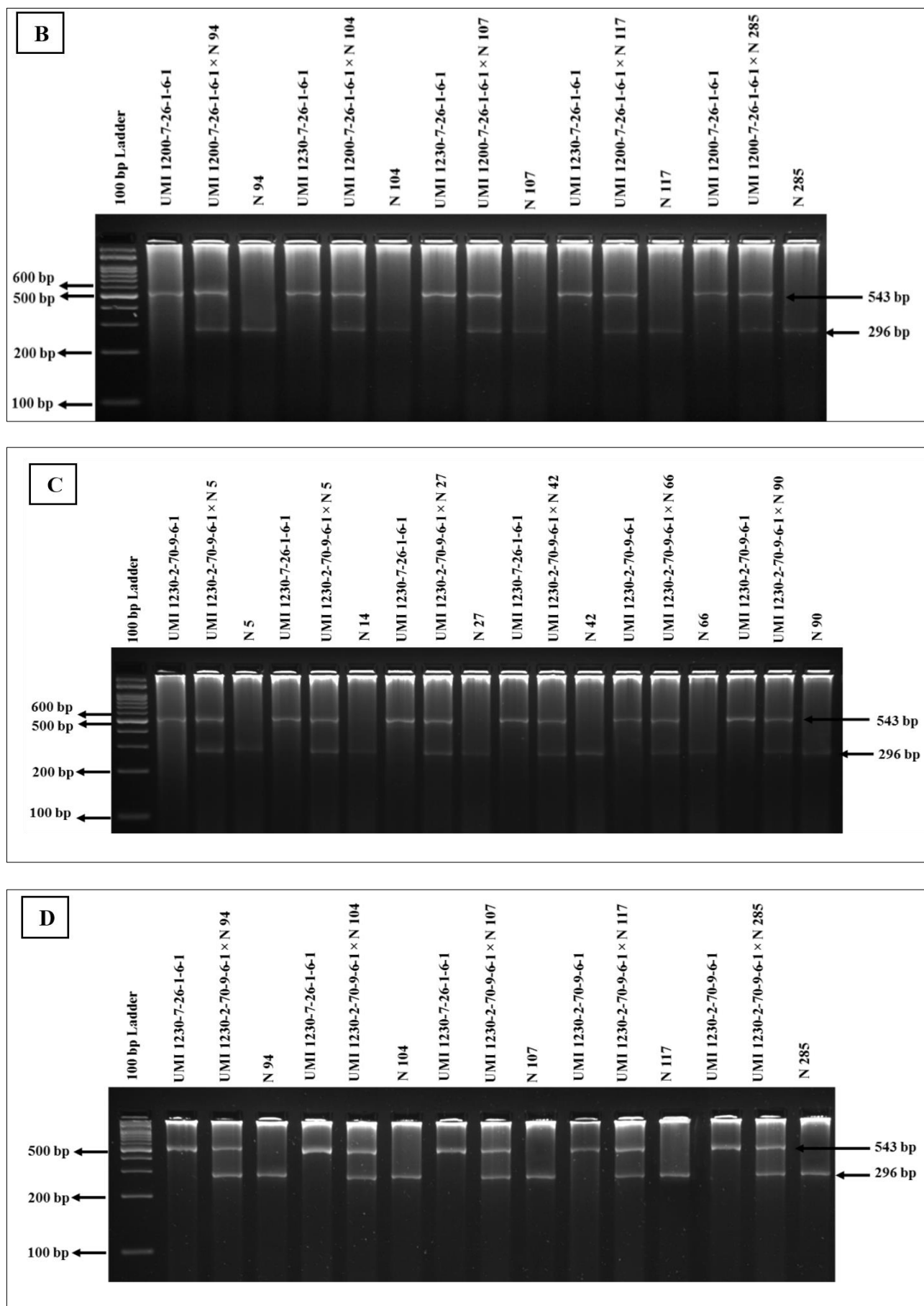


Fig 1: Confirmation of *crtRB1* gene specific marker in the parents and their hybrids; (A & B) UMI 1200-7-26-1-6-1 cross combinations and (C & D) UMI 1230-2-70-9-6-1 cross combinations

3.2. Dissection of gene action and combining ability

Analysis of Variance revealed highly significant differences among parents and hybrids for all 16 morphological and yield traits, validating the extensive genetic diversity present in the chosen germplasm (Table 1). Variance component analysis generated profound insights into gene action. As it was suggested higher GCA effects indicate additive type of gene action is responsible for the inheritance of the particular trait. Similarly, the estimates of GCA effects showed that the parents UMI 1230-2-70-9-6-1 among the testers and N 14, N 42, N 104, N 107 and N 285 among the lines possessed highly significant positive GCA effects for grain yield per plant indicating the presence of additive gene action (Table 2).

The estimates of SCA effects for hybrids for days to 50% tasselling and days to 50% silking only one cross N 117 × UMI 1200-7-26-1-6-1 recorded highly significant SCA effects. The hybrids N 14 × UMI 1200-7-26-1-6-1, N 66 × UMI 1200-7-26-1-6-1, N 94 × UMI 1200-7-26-1-6-1, N 117 × UMI 1200-7-26-1-6-1, N 285 × UMI 1200-7-26-1-6-1, N 5 × UMI 1230-2-70-

9-6-1, N 27 × UMI 1230-2-70-9-6-1, N 42 × UMI 1230-2-70-9-6-1, N 90 × UMI 1230-2-70-9-6-1, N 104 × UMI 1230-2-70-9-6-1 and N 107 × UMI 1230-2-70-9-6-1 recorded significant positive SCA effects for grain yield per plant (Table 3). Findings are found in accordance with (Kage *et al.*, 2013 and Premalatha, 2006). These hybrids could be used in heterosis breeding to exploit hybrid vigour for seed yield.

The Specific Combining Ability (SCA) variance vastly outweighed the General Combining Ability (GCA) variance for virtually all traits evaluated viz., for Grain yield, GCA variance = 3.00 vs. SCA variance = 513.21, for Cob weight GCA variance = 5.64 vs. SCA variance = 665.51 and for Plant height GCA variance = 2.71 vs. SCA variance = 646.38 (Table 4). The critically low GCA:SCA ratio like 0.012 for grain yield, statistically confirms the overwhelming predominance of non-additive gene action specifically dominance and epistatic interactions. This dictates that exploiting heterosis through the development of specific cross-combinations is the optimal path for yield improvement.

Table 1: Analysis of variance for different morphological traits

Source	Df	Mean sum of squares															
		DFT	DFS	PHT	LLT	LWD	LFC	TSL	BPT	CLT	CGT	CWT	NKR	KPR	HKW	GYP	SHP
Replication	1	0.51	2.80	1.15	0.26	0.0001	0.0006	0.25	0.0023	1.23	0.13	2.84	0.69	0.37	0.85	2.12	5.57
Genotype	34	15.54**	14.45**	2248.46**	372.09**	2.51**	316.16**	92.92**	4.69**	8.82**	7.03**	3690.16**	5.53**	37.03**	81.95**	2030.10**	71.89**
Error	34	0.24	0.24	1.05	3.51	0.02	2.54	0.38	0.10	0.23	0.23	1.49	0.21	2.99	22.30	1.68	1.83

*and**indicates significance at 5% and 1% level respectively

DFT	Days to 50% tasseling	TSL	Tassel length (cm)	KPR	Number of kernels per row
DFS	Days to 50% silking	BPT	Number of branches per tassel	HKW	100 kernel weight (g)
PHT	Plant height (cm)	CLT	Cob length (cm)	GYP	Grain yield per pant (g)
LLT	Leaf length (cm)	CGT	Cob girth (cm)	SHF	Shelling percentage
LWD	Leaf width (cm)	CWT	Cob weight (g)		
LFC	Length to first cob (cm)	NKR	Number of kernel rows per ear		

Table 2: The *gca* effects of parents for different traits

Parents	DFT	DFS	PHT	LLT	LWD	LFC	TSL	BPT	CLT	CGT	CWT	NKR	KPR	HGW	GYP	SHP
Lines																
UMI 1200-7-26-1-6-1	-0.34**	-0.32**	-16.17**	-7.55**	0.61**	-5.05**	-2.70**	-0.07	-0.62**	0.39 **	-4.09**	-0.24 *	-1.05**	0.64	-4.99 **	0.81 **
UMI 1230-2-70-9-6-1	0.34**	0.32**	16.17 **	7.55 **	-0.61**	5.05**	2.70**	0.07	0.62**	-0.39**	4.09 **	0.24*	1.05**	-0.64	4.99 **	-0.81 **
SE	0.09	0.10	0.25	0.22	0.02	0.34	0.14	0.07	0.09	0.08	0.27	0.09	0.17	0.45	0.29	0.13
Testers																
N5	0.66 **	0.14	-17.55**	-6.41**	-0.92**	-4.03**	-4.46**	-0.51 *	-2.18**	-1.10**	-17.30**	-0.20	-2.79 **	1.30	-18.17 **	-1.06 **
N14	-1.59 **	-2.11**	0.50	-2.51**	0.12	2.59**	0.24	0.94**	-0.24	-0.06	25.84 **	-0.17	0.28	6.55 **	23.81**	3.02 **
N 27	0.66 **	1.64 **	-6.75**	-5.71**	-0.30**	-1.08	-3.16**	1.32**	-0.53*	-0.72**	-25.81**	-0.45	-2.25 **	2.68 *	-22.30 **	-2.13 **
N 42	1.41 **	1.14 **	0.75	1.92 **	-0.01	-10.91**	-4.01**	-2.86 **	1.02**	0.73**	7.21 **	1.15 **	3.11 **	-2.13	19.10 **	0.75 *
N 66	0.16	0.39	12.88**	4.04 **	0.17*	-2.26 *	-1.86**	-1.21 **	-1.26**	0.20	-13.09**	0.34	-3.62 **	-3.71 **	-8.68 **	3.22 **
N 90	-1.09**	0.39	6.05**	-3.11**	-0.28**	-4.78**	2.14**	-0.11	0.10	-0.48*	-15.33**	-1.05**	0.55	-4.46 **	-2.98 **	7.05 **
N 94	0.91 **	0.64 *	7.75**	6.24 **	0.49**	9.57**	7.94**	0.59**	0.38	0.50 *	8.83 **	-1.00**	0.60	-0.75	-0.45	-0.34
N 104	1.66 **	0.14	-1.20	-2.21**	0.59**	2.14 *	-1.19**	0.89**	1.07**	0.75**	17.05**	2.82 **	-1.35 **	-1.21	7.97 **	-4.35 **
N 107	-0.34	-0.11	17.30**	8.17**	0.55**	6.57**	0.94**	-0.66 **	0.76**	0.12	5.81 **	-0.16	4.07 **	2.50 *	2.38 **	-4.96 **
N 117	-0.59 *	-0.86**	-14.05**	-2.21**	-0.00	-4.88**	0.44	1.02**	-0.41	1.05**	4.26 **	-0.50 *	0.50	1.51	-8.34 **	-5.60 **
N 285	-1.84 **	-1.36**	-5.67 **	1.79 **	-0.38**	7.07 **	2.99 **	0.59**	1.27**	-1.00**	2.53 **	-0.75**	0.90 *	-2.29 *	7.68 **	4.40 **
SE	0.23	0.25	0.59	0.51	0.06	0.81	0.32	0.18	0.23	0.19	0.63	0.22	0.40	1.05	0.69	0.031

*and**indicates significance at 5% and 1% level respectively

Table 3: The *sca* effects of hybrids for different traits

Hybrids	DFT	DFS	PHT	LLT	LWD	LFC	TSL	BPT	CLT	CGT	CWT	NKR	KPR	HGW	GYP	SHP
N 5 × UMI 1200-7-26-1-6-1	0.34	0.32	8.87 **	2.55 **	0.29 **	-1.9	3.40 **	-0.43	-0.86 *	-0.65 *	-23.58 **	-0.66 *	-2.16 **	-0.64	-20.35 **	0.35
N 14 × UMI 1200-7-26-1-6-1	0.59	0.57	3.92 **	4.05 **	-0.23 *	2.72 *	0.50	-0.78 **	-1.01 **	-0.11	36.04 **	-0.49	-0.92	10.13 **	33.08 **	6.19 **
N 27 × UMI 1200-7-26-1-6-1	-0.16	-0.18	21.57**	7.25 **	0.17	5.15 **	2.90 **	0.15	-0.18	-0.17	-15.15 **	-0.91 **	0.80	-0.37	-14.04 **	-4.87 **
N 42 × UMI 1200-7-26-1-6-1	-0.41	-0.68	-27.73 **	-3.83 **	-0.63 **	-9.32 **	-0.05	-0.98 **	0.36	0.20	-17.28 **	0.29	0.15	2.20	-4.33 **	-5.11 **
N 66 × UMI 1200-7-26-1-6-1	-0.16	0.57	-13.76 **	-10.40 **	-0.24 *	-0.28	1.90 **	-0.13	0.41	0.62 *	9.75 **	-0.42	1.47 *	-1.78	4.09 **	1.20 *
N 90 × UMI 1200-7-26-1-6-1	0.59	0.07	-21.58 **	-9.35 **	-0.08	-5.95 **	0.00	-1.63 **	-0.76 *	-0.09	1.71	0.29	-1.50 *	0.99	-3.13 **	0.97 *
N 94 × UMI 1200-7-26-1-6-1	0.59	-0.18	-18.73 **	-8.40 **	-0.41 **	10.50 **	-8.30 **	0.27	-0.13	-0.08	2.39 *	0.24	-0.35	-1.22	7.30 **	1.11 *
N 104 × UMI 1200-7-26-1-6-1	-0.66	0.32	2.02 *	5.35 **	0.20 *	3.82 **	-2.12 **	0.57 *	0.62	-0.11	-23.16 **	0.36	-2.40 **	-1.45	-20.88 **	-2.68 **
N 107 × UMI 1200-7-26-1-6-1	0.34	0.07	4.02 **	0.18	-0.16	-1.1	-2.60 **	1.02 **	0.41	0.67 *	6.23 **	2.68 **	1.59 *	-8.86 **	-5.74 **	-1.27 **
N 117 × UMI 1200-7-26-1-6-1	-1.41 **	-1.18 **	17.27 **	8.35 **	0.75 **	7.45 **	3.20 **	0.25	0.37	-0.46	10.11 **	-1.36 **	1.65 **	0.19	14.17 **	3.85 **

N 285 × UMI 1200-7-26-1-6-1	0.34	0.32	24.14 **	4.25 **	0.35 **	9.90 **	1.15 *	1.67 **	0.78 *	0.17	12.94 **	-0.01	1.67 **	0.8	9.83 **	0.26
N 5 × UMI 1230-2-70-9-6-1	-0.34	-0.32	-8.87 **	-2.55 **	-0.29 **	1.90	-3.40 **	0.43	0.86 *	0.65 *	23.58 **	0.66 *	2.16 **	0.64	20.35 **	-0.35
N 14 × UMI 1230-2-70-9-6-1	-0.59	-0.57	-3.92 **	-4.05 **	0.23 *	-2.72 *	-0.50	0.78 **	1.01 **	0.11	-36.04 **	0.49	0.92	-10.13 **	-33.08 **	-6.19 **
N 27 × UMI 1230-2-70-9-6-1	0.16	0.18	-21.57 **	-7.25 **	-0.17	-5.15 **	-2.90 **	-0.15	0.18	0.17	15.15 **	0.91 **	-0.80	0.37	14.04 **	4.87 **
N 42 × UMI 1230-2-70-9-6-1	0.41	0.68	27.73 **	3.83 **	0.63 **	9.32 **	0.05	0.98 **	-0.36	-0.20	17.28 **	-0.29	-0.15	-2.20	4.33 **	5.11 **
N 66 × UMI 1230-2-70-9-6-1	0.16	-0.57	13.76 **	10.40 **	0.24 *	0.28	-1.90 **	0.13	-0.41	-0.62 *	-9.75 **	0.42	-1.47 *	1.78	-4.09 **	-1.20 *
N 90 × UMI 1230-2-70-9-6-1	-0.59	-0.07	21.58 **	9.35 **	0.08	5.95 **	-0.00	1.63 **	0.76 *	0.09	-1.71	-0.29	1.50 *	-0.99	3.13 **	-0.97 *
N 94 × UMI 1230-2-70-9-6-1	-0.59	0.18	18.73 **	8.40 **	0.41 **	10.50 **	8.30 **	-0.27	0.13	0.08	-2.39 *	-0.24	0.35	1.22	-7.30 **	-1.11 *
N 104 × UMI 1230-2-70-9-6-1	0.66	-0.32	-2.02 *	-5.35 **	-0.20 *	-3.82 **	2.12 **	-0.57 *	-0.62	0.11	23.16 **	-0.36	2.40 **	1.45	20.88 **	2.68 **
N 107 × UMI 1230-2-70-9-6-1	-0.34	-0.07	-4.02 **	-0.18	0.16	1.10	2.60 **	-1.02 **	-0.41	-0.67 *	-6.23 **	-2.68 **	-1.59 *	8.86 **	5.74 **	1.27 *
N 117 × UMI 1230-2-70-9-6-1	1.41 **	1.18 **	-17.27 **	-8.35 **	-0.75 **	-7.45 **	-3.20 **	-0.25	-0.37	0.46	-10.11 **	1.36 **	-1.65 **	-0.19	-14.17 **	-3.85 **
N 285 × UMI 1230-2-70-9-6-1	-0.34	-0.32	-24.14 **	-4.25 **	-0.35 **	-9.90 **	-1.15 *	-1.67 **	-0.78 *	-0.17	-12.94 **	0.01	-1.67 **	-0.8	-9.83 **	-0.26
SE	0.32	0.36	0.84	0.73	0.09	1.15	0.46	0.25	0.33	0.26	0.89	0.31	0.57	1.49	0.97	0.45

*and** indicates significance at 5% and 1% level respectively

Table 4: Magnitude of genetic variance for different traits

Sl. No.	Characters	GCA variance	SCA variance	$\sigma^2 A$	$\sigma^2 D$	$\sigma^2 A / \sigma^2 D$
1.	Days to 50% tasseling	0.06	0.70	0.12	0.70	0.171
2.	Days to 50% silking	0.05	0.44	0.1	0.44	0.227
3.	Plant height	2.71	646.38	5.42	646.38	0.008
4.	Leaf length	1.96	94.00	3.92	94.00	0.042
5.	Leaf width	0.02	0.30	0.04	0.30	0.133
6.	Length to first cob	1.06	86.58	2.12	86.58	0.024
7.	Length of tassel	0.47	22.89	0.94	22.89	0.041
8.	Number of branches/ tassel	0.03	1.67	0.06	1.67	0.036
9.	Cob length	0.06	0.68	0.12	0.68	0.176
10.	Cob girth	0.03	0.24	0.06	0.24	0.250
11.	Cob weight	5.64	665.51	11.28	665.51	0.017
12.	Number of kernel rows/ cob	0.006	2.11	0.012	2.11	0.006
13.	Number of kernels/ row	0.23	4.56	0.46	4.56	0.101
14.	100 grain weight	0.60	36.72	1.2	36.72	0.033
15.	Grain yield per plant	3.00	513.21	6	513.21	0.012
16.	Shelling percentage	0.45	22.91	0.9	22.91	0.039

Table 5: Standard heterosis for different morphological characters

Hybrids	Days to 50% tasseling		Days to 50% silking		Plant height		Leaf length	
	diii (a)	diii(b)	diii (a)	diii(b)	diii (a)	diii(b)	diii (a)	diii(b)
N 5 × UMI 1200-7-26-1-6-1	6.93 **	5.88 **	7.62 **	5.61 **	-20.21 **	-4.53 **	-10.52 **	-5.62 **
N 14 × UMI 1200-7-26-1-6-1	2.97 **	1.96 *	3.81 **	1.87	-13.42 **	3.60 **	-2.74	2.58
N 27 × UMI 1200-7-26-1-6-1	5.94 **	4.90 **	9.52 **	7.48 **	-8.03 **	10.04 **	-2.74	2.58
N 42 × UMI 1200-7-26-1-6-1	6.93 **	5.88 **	7.62 **	5.61 **	-29.69 **	-15.87 **	-7.71 **	-2.66
N 66 × UMI 1200-7-26-1-6-1	4.95 **	3.92 **	8.57 **	6.54 **	-16.17 **	0.31	-14.12 **	-9.42 **
N 90 × UMI 1200-7-26-1-6-1	3.96 **	2.94 **	7.62 **	5.61 **	-23.76 **	-8.77 **	-22.91 **	-18.69 **
N 94 × UMI 1200-7-26-1-6-1	7.92 **	6.86 **	7.62 **	5.61 **	-21.40 **	-5.95 **	-8.07 **	-3.04
N 104 × UMI 1200-7-26-1-6-1	6.93 **	5.88 **	7.62 **	5.61 **	-15.28 **	1.36	-0.43	5.02 **
N 107 × UMI 1200-7-26-1-6-1	4.95 **	3.92 **	6.67 **	4.67 **	-4.66 **	14.07 **	7.06 **	12.92 **
N 117 × UMI 1200-7-26-1-6-1	0.99	0.00	2.86 *	0.93	-14.04 **	2.85 **	3.89 *	9.57 **
N 285 × UMI 1200-7-26-1-6-1	1.98 *	0.98	4.76 **	2.80 *	-6.14 **	12.31 **	3.75 *	9.42 **
N 5 × UMI 1230-2-70-9-6-1	6.93 **	5.88 **	7.62 **	5.61 **	-12.64 **	4.53 **	3.89 *	9.57 **
N 14 × UMI 1230-2-70-9-6-1	1.98 *	0.98	2.86 *	0.93	-0.73	18.78 **	7.35 **	13.22 **
N 27 × UMI 1230-2-70-9-6-1	7.92 **	6.86 **	11.43 **	9.35 **	-13.63 **	3.35 **	-1.87	3.50 *
N 42 × UMI 1230-2-70-9-6-1	9.90 **	8.82 **	11.43 **	9.35 **	15.80 **	38.56 **	25.07 **	31.91 **
N 66 × UMI 1230-2-70-9-6-1	6.93 **	5.88 **	7.62 **	5.61 **	14.84 **	37.41 **	37.61 **	45.14 **
N 90 × UMI 1230-2-70-9-6-1	2.97 **	1.96 *	8.57 **	6.54 **	15.36 **	38.03 **	25.79 **	32.67 **
N 94 × UMI 1230-2-70-9-6-1	6.93 **	5.88 **	9.52 **	7.48 **	14.77 **	37.32 **	37.90 **	45.44 **
N 104 × UMI 1230-2-70-9-6-1	10.89 **	9.80 **	7.62 **	5.61 **	-0.62	18.91 **	5.91 **	11.70 **
N 107 × UMI 1230-2-70-9-6-1	4.95 **	3.92 **	7.62 **	5.61 **	7.93 **	29.14 **	28.31 **	35.33 **
N 117 × UMI 1230-2-70-9-6-1	7.92 **	6.86 **	8.57 **	6.54 **	-15.18 **	1.49	1.59	7.14 **
N 285 × UMI 1230-2-70-9-6-1	1.98 *	0.98	4.76 **	2.80 *	-14.40 **	2.42 **	13.26 **	19.45 **
SE(d)	0.46	0.46	0.56	0.56	1.26	1.26	1.07	1.07

*and** indicates significance at 5% and 1% level respectively

diii (a) and diii (b) – indicates standard heterosis over the CO 6 and NK 6240 respectively

Table 6: Standard heterosis for different morphological characters (contd.,)

Hybrids	Leaf width		Length to first cob		Length of tassel		Number of branches per tassel	
	diii (a)	diii(b)	diii (a)	diii(b)	diii (a)	diii(b)	diii (a)	diii(b)
N 5 × UMI 1200-7-26-1-6-1	-8.68 **	-7.47 **	-32.53 **	-26.35 **	-1.77	1.83	-5.21	7.06
N 14 × UMI 1200-7-26-1-6-1	-1.84	-0.53	-17.53 **	-9.97 **	3.54	7.34 **	6.25	20.00 **
N 27 × UMI 1200-7-26-1-6-1	-2.11	-0.80	-19.20 **	-11.79 **	0.59	4.28 *	19.79 **	35.29 **

N 42 × UMI 1200-7-26-1-6-1	-8.82 **	-7.60 **	-51.60 **	-47.16 **	10.62 **	-7.34 **	-35.42 **	-27.06 **
N 66 × UMI 1200-7-26-1-6-1	-1.32	0.06	-28.00 **	-21.40 **	1.47	5.20 *	-9.37 *	2.35
N 90 × UMI 1200-7-26-1-6-1	-5.13 **	-3.87 *	-38.93 **	-33.33 **	7.67 **	11.62 **	-13.54 **	-2.35
N 94 × UMI 1200-7-26-1-6-1	0.66	2.00	-25.87 **	-19.07 **	0.29	3.98	13.54 **	28.24 **
N 104 × UMI 1200-7-26-1-6-1	10.00 **	11.47 **	-16.67 **	-9.02 **	-8.41 **	-5.05 *	19.79 **	35.29 **
N 107 × UMI 1200-7-26-1-6-1	4.74 *	6.13 **	-17.33 **	-9.75 **	-3.54	0.12	8.33 *	22.35 **
N 117 × UMI 1200-7-26-1-6-1	9.47 **	10.93 **	-21.20 **	-13.97 **	12.09 **	16.21 **	17.71 **	32.94 **
N 285 × UMI 1200-7-26-1-6-1	-0.79	0.53	-2.00	6.99 **	13.57 **	17.74 **	28.12 **	44.71 **
N 5 × UMI 1230-2-70-9-6-1	-0.26	1.07	-14.00 **	-6.11 *	-5.90 **	-2.45	5.21	18.82 **
N 14 × UMI 1230-2-70-9-6-1	20.39 **	22.00 **	-11.33 **	-3.20	16.52 **	20.80 **	23.96 **	40.00 **
N 27 × UMI 1230-2-70-9-6-1	9.47 **	10.93 **	-19.47 **	-12.08 **	-0.59	3.06	18.23 **	33.53 **
N 42 × UMI 1230-2-70-9-6-1	23.82 **	25.47 **	-13.27 **	-5.31 *	5.60 **	9.48 **	-13.54 **	-2.35
N 66 × UMI 1230-2-70-9-6-1	21.12 **	22.73 **	-13.80 **	-5.90 *	6.19 **	10.09 **	-5.21	7.06
N 90 × UMI 1230-2-70-9-6-1	13.03 **	14.53 **	-9.60 **	-1.31	23.60 **	28.13 **	21.88 **	37.65 **
N 94 × UMI 1230-2-70-9-6-1	27.63 **	29.33 **	15.60 **	26.20 **	65.19 **	71.25 **	9.38 *	23.53 **
N 104 × UMI 1230-2-70-9-6-1	20.92 **	22.53 **	-13.40 **	-5.46 *	20.06 **	24.46 **	9.38 *	23.53 **
N 107 × UMI 1230-2-70-9-6-1	25.00 **	26.67 **	-0.93	8.15 **	27.73 **	32.42 **	-11.46 **	0.42
N 117 × UMI 1230-2-70-9-6-1	5.79 **	7.20 **	-27.60 **	-20.96 **	9.14 **	13.15 **	14.06 **	28.82 **
N 285 × UMI 1230-2-70-9-6-1	6.05 **	7.47 **	-14.93 **	-7.13 **	22.71 **	27.22 **	-5.21	7.06
SE _(d)	0.13	0.13	1.59	1.59	0.66	0.66	0.35	0.35

*and**indicates significance at 5% and 1% level respectively

diii (a) and diii (b) – indicates standard heterosis over the CO 6 and NK 6240 respectively

Table 7: Standard heterosis for different morphological characters (contd.,)

Hybrids	Cob length		Cob girth		Cob weight		Number of kernel rows per ear	
	diii (a)	diii(b)	diii (a)	diii(b)	diii (a)	diii(b)	diii (a)	diii(b)
N 5 × UMI 1200-7-26-1-6-1	-11.15 *	-24.60 **	-8.63 **	-1.11	-43.38 **	-27.81 **	-8.50 *	15.70 **
N 14 × UMI 1200-7-26-1-6-1	1.98	-13.45 **	2.92	11.39 **	19.83 **	52.79 **	-7.19 *	17.36 **
N 27 × UMI 1200-7-26-1-6-1	6.02	-10.02 *	-2.34	5.70	-43.43 **	-27.88 **	-11.76 **	11.57 *
N 42 × UMI 1200-7-26-1-6-1	21.28 **	2.93	10.96 **	20.09 **	-24.43 **	-3.65 **	6.54	34.71 **
N 66 × UMI 1200-7-26-1-6-1	4.99	-10.90 *	10.16 **	19.22 **	-20.28 **	1.64	-3.33	22.23 **
N 90 × UMI 1200-7-26-1-6-1	6.38	-9.71 *	0.46	8.23 *	-26.61 **	-6.42 **	-7.84 *	16.53 **
N 94 × UMI 1200-7-26-1-6-1	12.99 *	-4.11	7.24 *	16.06 **	-11.33 **	13.06 **	-7.84 *	16.53 **
N 104 × UMI 1200-7-26-1-6-1	23.62 **	4.92	8.85 **	17.80 **	-21.99 **	-0.53	17.97 **	49.17 **
N 107 × UMI 1200-7-26-1-6-1	19.74 **	1.62	9.94 **	18.99 **	-10.82 **	13.70 **	13.66 **	43.72 **
N 117 × UMI 1200-7-26-1-6-1	10.93 *	-5.85	8.48 **	17.41 **	-9.39 **	15.53 **	-15.03 **	7.44
N 285 × UMI 1200-7-26-1-6-1	26.19 **	7.10	-1.90	6.17	-8.72 **	16.39 **	-7.84 *	16.53 **
N 5 × UMI 1230-2-70-9-6-1	10.64 *	-6.10	-4.75	3.09	-9.33 **	15.60 **	3.27	30.58 **
N 14 × UMI 1230-2-70-9-6-1	25.97 **	6.91	-1.21	6.92 *	-19.47 **	2.67 *	2.39	29.46 **
N 27 × UMI 1230-2-70-9-6-1	17.75 **	-0.06	-5.56	2.22	-19.75 **	2.32 *	3.27	30.58 **
N 42 × UMI 1230-2-70-9-6-1	25.17 **	6.23	2.34	10.76 **	1.87 *	29.89 **	5.88	33.88 **
N 66 × UMI 1230-2-70-9-6-1	8.07	-8.28	-4.61	3.24	-27.25 **	-7.24 **	5.23	33.06 **
N 90 × UMI 1230-2-70-9-6-1	26.63 **	7.47	-4.39	3.48	-23.68 **	-2.69 *	-8.50 *	15.70 **
N 94 × UMI 1230-2-70-9-6-1	24.06 **	5.29	2.78	11.23 **	-9.23 **	15.73 **	-7.84 *	16.53 **
N 104 × UMI 1230-2-70-9-6-1	23.62 **	4.92	4.82	13.45 **	11.54 **	42.22 **	16.34 **	47.11 **
N 107 × UMI 1230-2-70-9-6-1	22.89 **	4.30	-5.56	2.22	-13.45 **	10.35 **	-18.30 **	3.31
N 117 × UMI 1230-2-70-9-6-1	14.60 **	-2.74	9.50 **	18.51 **	-16.79 **	6.09 **	5.88	33.88 **
N 285 × UMI 1230-2-70-9-6-1	23.92 **	5.17	-10.05 **	-2.65	-19.60 **	2.51 *	-4.58	20.66 **
SE _(d)	0.65	0.65	0.40	0.40	1.31	1.31	0.52	0.52

*and**indicates significance at 5% and 1% level respectively

diii (a) and diii (b) – indicates standard heterosis over the CO 6 and NK 6240 respectively

Table 8: Standard heterosis for different morphological characters (contd.,)

Hybrids	Number of kernels per row		100 grain weight		Grain yield per pant		Shelling percentage	
	diii (a)	diii(b)	diii (a)	diii(b)	diii (a)	diii(b)	diii (a)	diii(b)
N 5 × UMI 1200-7-26-1-6-1	-9.19	-30.86 **	-20.25 **	-4.97	-55.40 **	-46.85 **	-7.79 **	-7.19 **
N 14 × UMI 1200-7-26-1-6-1	10.65 *	-15.75 **	-14.47 *	1.93	26.73 **	51.01 **	5.86 **	6.55 **
N 27 × UMI 1200-7-26-1-6-1	6.91	-18.60 **	-16.90 **	-0.97	-53.52 **	-44.61 **	-16.43 **	-15.89 **
N 42 × UMI 1200-7-26-1-6-1	28.62 **	2.07	-21.44 **	-6.38	-9.53 **	7.81 **	-12.80 **	-12.24 **
N 66 × UMI 1200-7-26-1-6-1	3.73	-21.02 **	-32.76 **	-19.87 **	-26.19 **	-12.05 **	-0.74	-0.09
N 90 × UMI 1200-7-26-1-6-1	9.22	-16.84 **	-28.67 **	-15.00 *	-27.50 **	-13.61 **	4.23 **	4.91 **
N 94 × UMI 1200-7-26-1-6-1	14.75 **	-12.63 **	-25.60 **	-11.34	-16.35 **	-0.31	-5.75 **	-5.14 **
N 104 × UMI 1200-7-26-1-6-1	-3.69	-26.67 **	-27.00 **	-13.01	-33.36 **	-20.59 **	-16.47 **	-15.93 **
N 107 × UMI 1200-7-26-1-6-1	39.68 **	6.35	-34.52 **	-21.97 **	-25.13 **	-10.79 **	-15.38 **	-14.83 **
N 117 × UMI 1200-7-26-1-6-1	23.50 **	-5.96	-18.14 **	-2.45	-17.22 **	-1.36	-9.22 **	-8.63 **
N 285 × UMI 1200-7-26-1-6-1	25.44 **	-4.49	-24.63 **	-10.19	-7.17 **	10.62 **	-0.40	0.25
N 5 × UMI 1230-2-70-9-6-1	20.37 **	-8.35 *	-20.27 **	-4.99	-11.77 **	5.14 *	-3.81 **	-3.18 **

N 14 × UMI 1230-2-70-9-6-1	28.78 **	-1.95	-31.50 **	-18.37 *	-21.63 **	-6.61 **	-6.22 **	-5.61 **
N 27 × UMI 1230-2-70-9-6-1	9.22	-16.84 **	-18.01 **	-2.29	-20.76 **	-5.57 **	1.90 *	2.56 **
N 42 × UMI 1230-2-70-9-6-1	36.87 **	4.21	-33.02 **	-20.18 **	6.52 **	26.94 **	6.20 **	6.89 **
N 66 × UMI 1230-2-70-9-6-1	-0.18	-24.00 **	-28.14 **	-14.37 *	-24.63 **	-10.19 **	0.91	1.56
N 90 × UMI 1230-2-70-9-6-1	32.72 **	1.05	-35.30 **	-22.90 **	-13.52 **	3.05	6.50 **	7.19 **
N 94 × UMI 1230-2-70-9-6-1	27.65 **	-2.81	-23.26 **	-8.55	-20.32 **	-5.05 *	-3.86 **	-3.24 **
N 104 × UMI 1230-2-70-9-6-1	28.11 **	-2.46	-23.73 **	-9.11	11.19 **	32.50 **	-4.15 **	-3.53 **
N 107 × UMI 1230-2-70-9-6-1	34.68 **	2.54	-1.12	17.83 *	-6.65 **	11.24 **	-6.94 **	-6.34 **
N 117 × UMI 1230-2-70-9-6-1	17.97 **	-10.18 *	-21.53 **	-6.49	-33.02 **	-20.18 **	-14.86 **	-14.30 **
N 285 × UMI 1230-2-70-9-6-1	19.75 **	-8.82 *	-30.50 **	-17.18 *	-15.50 **	0.70	3.82 **	4.49 **
SE _(d)	1.07	1.07	2.76	2.76	1.78	1.78	0.60	0.60

*and**indicates significance at 5% and 1% level respectively diii (a) and diii (b) – indicates standard heterosis over the CO 6 and NK 6240 respectively

Table 9: Mean performances of parents for different traits

PARENTS	DFT	DFS	PHT	LLT	LWD	LFC	TSL	BPT	CLT	CGT	CWT	NKR	KPR	HGW	GYP	SHP
Lines																
UMI 1200- 7-26-1-6-1	57.00*	60.00*	122	47.80	5.40	28.90	24.70	9.50	9.53	10.32	53.68	15.40*	16.94*	34.22*	42.12*	70.13*
UMI 1230-2-70-9-6-1	60.00	63.00	148.60*	54.70*	6.45*	62.90	24.70	9.50	9.70	11.66*	54.68	12.80	15.70	28.93	38.85	64.04
Mean	58.50	61.5	135.3	51.25	5.92	45.90	24.70	9.50	9.61	10.98	54.18	14.10	16.32	31.57	40.48	67.08
CD @ 5%	0.28	0.32	0.74	0.64	0.08	1.02	0.41	0.23	0.29	0.23	0.79	0.28	0.51	1.32	0.86	0.40
Testers																
N5	59.50	62.50	139.35	50.30	6.66	56.10	26.30	7.70	13.09	8.47	61.89	13.78*	13.78	38.95*	44.70	69.08
N14	58.50*	61.50*	118.10	48.30	6.53	43.00	24.70	8.40	13.41	11.38*	120.68*	13.55	22.81*	43.62*	83.71*	69.24
N 27	58.50*	61.50*	153.70	58.60*	6.26	52.00	23.40	8.70	12.23	8.09	47.16	11.99	22.29*	42.05*	28.23	60.44
N 42	58.50*	61.50*	142.30	70.30*	7.48*	43.50	36.10*	6.50	13.16	10.90	67.66*	12.43	19.27	38.96*	45.56	68.69
N 66	57.50*	60.50*	119.50	47.80	7.25*	54.50	29.70*	7.30	12.02	9.76	39.24	12.79	13.88	21.66	23.51	54.65
N 90	58.50*	61.00*	114.70	48.40	6.85	38.30	26.20	11.40*	14.77	12.90*	59.18	14.00*	21.60*	26.74	43.18	63.55
N 94	58.50*	61.50*	104.80	50.90	6.20	36.40	27.95	9.45	14.26	11.75*	77.74*	12.32	22.35	27.13	55.69	78.93*
N 104	59.00	61.00*	151.40	58.80*	6.32	58.00	36.10*	9.70*	12.18	10.97	62.72	15.00*	23.60*	27.96	44.13	71.78*
N 107	58.50*	61.50*	142.80	61.40*	6.41	63.30	28.91	9.15	13.07	11.09	77.72*	12.90	22.39*	35.57	57.07	76.29*
N 117	59.00	62.00	105.20	51.20	6.03	31.60	23.32	10.70*	12.73	11.03	48.21	13.26	20.21	27.57	28.86	60.77
N 285	57.50*	60.50*	118.05	52.65	6.30	52.50	28.10	10.50*	13.72	10.83	43.74	11.38*	20.23	30.40	32.43	72.42*
Mean	58.50	62.50	128.26	54.42	6.57	48.10	28.25	9.04	13.14	10.65	64.17	13.03	20.21	32.78	44.27	69.08
CD @ 5%	0.67	0.75	1.74	1.51	0.19	2.40	0.96	0.54	0.68	0.55	1.87	0.66	1.20	3.10	2.03	0.98

*and**indicates significance at 5% and 1% level respectively

Table 10: Mean performances of hybrids for different traits

HYBRIDS	DFT	DFS	PHT	LLT	LWD	LFC	TSL	BPT	CLT	CGT	CWT	NKR	KPR	HGW	GYP	SHP
N 5 × UMI 1200-7-26-1-6-1	54.00*	56.50*	154.00	62.10	6.94	50.60	33.30	9.10	12.11	12.50	92.03	14.00	19.70	39.22	51.82	67.06
N 14 × UMI 1200-7-26-1-6-1	52.00*	54.50*	167.10	67.50	7.46	61.85	35.10	10.20	13.90	14.08	194.79*	14.20	24.01	42.06	147.23*	76.99 *
N 27 × UMI 1200-7-26-1-6-1	53.50*	57.50	177.50	67.50	7.44	60.60	34.10	11.50*	14.45	13.36	91.95	13.50	23.20	40.87	54.00	60.77
N 42 × UMI 1200-7-26-1-6-1	54.00*	56.50*	135.70	64.05	6.93	36.30	30.30	6.20	16.53	15.18*	122.84	16.30	27.91*	38.63	105.10*	63.41
N 66 × UMI 1200-7-26-1-6-1	53.00*	57.00*	161.80	59.60	7.50	54.00	34.40	8.70	14.31	15.07*	129.58	14.79	22.51	33.07	85.75	72.19
N 90 × UMI 1200-7-26-1-6-1	52.50*	56.50*	147.15	53.50	7.21	45.80	36.50	8.30	14.50	13.68	119.30	14.10	23.70	35.08	84.23	75.80
N 94 × UMI 1200-7-26-1-6-1	54.50*	56.50*	151.70	63.80	7.65	55.60	34.00	10.90*	15.40	14.67	144.14	14.10	24.90	36.59	97.19	68.53
N 104 × UMI 1200-7-26-1-6-1	54.00*	56.50*	163.50	69.10	8.36	62.50	31.05	11.50*	16.85	14.89*	126.81	18.05*	20.90	35.90	77.42	60.74
N 107 × UMI 1200-7-26-1-6-1	53.00*	56.00*	184.00*	74.30	7.96	62.00	32.70	10.40*	16.32	15.04	144.96*	17.39*	30.31*	32.20	86.98	61.53
N 117 × UMI 1200-7-26-1-6-1	51.00*	54.00*	165.90	72.10	8.32	59.10	38.00	11.30*	15.12	14.84*	147.29*	13.00	26.80	40.26	96.17	66.02
N 285 × UMI 1200-7-26-1-6-1	51.50*	55.00*	181.15	72.00	7.54	73.50	38.50*	12.30*	17.20*	13.42	148.38*	14.10	27.22	37.07	107.85*	72.43
N 5 × UMI 1230-2-70-9-6-1	54.00*	56.50*	168.60	72.10	7.58	64.50	31.90	10.10	15.08	13.03	147.38*	15.80	26.12	39.21	102.51*	69.95
N 14 × UMI 1230-2-70-9-6-1	51.50*	54.00*	191.60*	74.50	9.15*	66.50	39.50*	11.90*	17.17*	13.52	130.90	15.66	27.94*	33.69	91.05	68.19
N 27 × UMI 1230-2-70-9-6-1	54.50	58.50	166.70	68.10	8.32	60.40	33.70	11.35*	16.05	12.92	130.44	15.80	23.70	40.33	92.06	74.10*
N 42 × UMI 1230-2-70-9-6-1	55.50*	58.50	223.50*	86.80*	9.41*	65.05	35.80	8.30	17.06	14.00	165.60*	16.20*	29.70*	32.94	123.76*	77.23*
N 66 × UMI 1230-2-70-9-6-1	54.00*	56.50*	221.65*	95.50*	9.20*	64.65	36.00	9.10	14.73	13.05	118.26	16.10	21.66	35.34	87.56	73.38*
N 90 × UMI 1230-2-70-9-6-1	52.00*	57.00*	222.65*	87.30*	8.59	67.80 *	41.90*	11.70*	17.26*	13.08	124.06	14.00	28.80*	31.82	100.47*	77.44*
N 94 × UMI 1230-2-70-9-6-1	54.00*	57.50	221.50*	95.70*	9.70*	86.70 *	56.00	10.50	16.91	14.06	147.54*	14.10	27.70	37.74	92.57	69.91
N 104 × UMI 1230-2-70-9-6-1	56.00	56.50*	191.80*	73.50	9.19*	64.95	40.70*	10.50	16.85	14.34	181.32*	17.80*	27.80	37.51	129.18*	69.70
N 107 × UMI 1230-2-70-9-6-1	53.00*	56.50*	208.30*	89.05*	9.50*	74.30 *	43.30*	8.50	16.75	12.92	140.69*	12.50	29.23	48.63*	108.46*	67.67
N 117 × UMI 1230-2-70-9-6-1	54.50	57.00*	163.70	70.50	8.04	54.30	37.00	10.95*	15.62	14.98*	135.25	16.20	25.60	38.59	77.82	61.92
N 285 × UMI 1230-2-70-9-6-1	51.50*	55.00*	165.20	78.60*	8.06	63.80	41.60*	9.10	16.89	12.30	130.69	14.60	25.98	34.18	98.17	75.49*
Mean	53.12	56.08	178.85	73.51	8.18	61.58	37.06	10.11	15.78	13.86	137.01	15.10	25.70	37.32	95.33	68.80
CD @ 5%	0.95	1.16	2.62	2.22	0.27	3.30	1.37	0.73	1.36	0.83	2.72	1.09	2.21	5.73	3.69	1.26

*and**indicates significance at 5% and 1% level respectively

Table 11: Phenotypic (P) and Genotypic (G) correlation coefficient for different traits

Character		DFS	PHT	LLT	LWD	LFC	TSL	BPT	CLT	CGT	CWT	NKR	KPR	SHP	HGW	GYP
DFT	P	0.9475**	-0.6083	-0.6071	-0.6188	-0.4697	-0.6443	-0.3555	-0.6626	-0.7062	-0.7424	-0.3569	-0.6235	-0.1918	-0.298	-0.7085
	G	0.9779**	-0.6194	-0.6173	-0.6339	-0.4824	-0.6579	-0.3594	-0.6862	-0.729	-0.7543	-0.3709	-0.6788	-0.182	-0.4506	-0.7209
DFS	P		-0.5908	-0.5961	-0.6343	-0.4568	-0.6285	-0.3579	-0.6668	-0.745	-0.7972	-0.4676	-0.6458	-0.1357	-0.3252	-0.7552
	G		-0.6013	-0.6111	-0.6571	-0.4674	-0.6455	-0.3677	-0.6879	-0.7825	-0.8117	-0.4917	-0.686	-0.1345	-0.4219	-0.768
PHT	P			0.9239**	0.862**	0.813**	0.7581**	0.2503	0.6156**	0.4684*	0.662**	0.3932*	0.6151**	0.2447	0.2968*	0.644**

	G			0.933**	0.8718**	0.8205**	0.7614**	0.2569	0.6311**	0.4861*	0.6628**	0.4093**	0.6694**	0.2514	0.4325**	0.6454**
LLT	P				0.8759**	0.7433**	0.7974**	0.1914	0.6781**	0.4894*	0.6485**	0.3404*	0.6509**	0.2471	0.3128*	0.637**
	G				0.882**	0.7513**	0.8081**	0.201	0.6998**	0.5096**	0.6549**	0.3606*	0.7117**	0.2515	0.4423*	0.644**
LWD	P					0.7292**	0.7991**	0.2307	0.7698**	0.5865**	0.7206**	0.4289*	0.608**	0.1236	0.257	0.6811**
	G					0.7352**	0.8075**	0.2286	0.7916**	0.6076**	0.7277**	0.4474*	0.658**	0.1358	0.371*	0.6883**
LFC	P						0.7131**	0.3314*	0.5155**	0.3017*	0.5358**	0.1679	0.4385*	0.1717	0.2201	0.4945**
	G						0.7205**	0.3383*	0.53**	0.3156*	0.541**	0.1763	0.4765*	0.1846	0.354*	0.499**
TSL	P							0.2153	0.6505**	0.5119**	0.6309**	0.2151	0.6022**	0.2901	0.2173	0.5958**
	G							0.2216	0.6658**	0.532**	0.6333**	0.2227	0.6544**	0.3005*	0.3388*	0.5977**
BPT	P								0.3357*	0.3611*	0.2679	0.2075	0.3148*	0.0127	-0.0497	0.1814
	G								0.354*	0.3701*	0.2739	0.1969	0.3299*	0.0118	-0.0739	0.1858
CLT	P									0.6596**	0.7309**	0.4518*	0.7839**	0.2018	0.2098	0.7202**
	G									0.6938**	0.7479**	0.472*	0.8419**	0.2059	0.3159*	0.7357**
CGT	P										0.7791**	0.5811**	0.6533**	0.0783	0.1699	0.713**
	G										0.8035**	0.596**	0.7052**	0.0776	0.2	0.7361**
CWT	P											0.5438**	0.7358**	0.3281*	0.4148*	0.9689**
	G											0.5659**	0.7943**	0.3351*	0.5993**	0.9696**
NKR	P												0.3808*	-0.0268	0.0326*	0.4968**
	G												0.3961*	-0.0325	0.0715	0.5137**
KPR	P													0.2526	0.3749*	0.7304**
	G													0.2861	0.2615	0.7889**
HGW	P														0.0555	0.4356**
	G														0.1047	0.4451**
GYP	P															0.393*
	G															0.5795**

Genotypic residual effect = 0.1009

G: Genotypic correlation coefficient

Table 12: Genotypic (G) path coefficient for different traits

Traits		DFT	DFS	PHT	LLT	LWD	LFC	TSL	BPT	CLT	CGT	CWT	NKR	KPR	SHF	HGW	GYP
DFT	G	3.7246	-3.8722	-1.0943	0.4687	0.5532	0.1278	0.0317	0.0753	-0.7509	-0.2672	-0.0087	0.233	0.2281	-0.057	-0.1131	-0.7209
DFS	G	3.6422	-3.9598	-1.0624	0.4639	0.5735	0.1239	0.0311	0.0771	-0.7527	-0.2868	-0.0094	0.3089	0.2305	-0.0421	-0.1059	-0.768
PHT	G	-2.3069	2.3809	1.7669	-0.7083	-0.7608	-0.2174	-0.0367	-0.0538	0.6907	0.1781	0.0077	-0.2572	-0.225	0.0787	0.1086	0.6455**
LLT	G	-2.2994	2.4198	1.6485	-0.7592	-0.7697	-0.1991	-0.039	-0.0421	0.7658	0.1867	0.0076	-0.2266	-0.2392	0.0788	0.111	0.644**
LWD	G	-2.3611	2.6022	1.5403	-0.6696	-0.8727	-0.1948	-0.039	-0.0479	0.8663	0.2227	0.0084	-0.2811	-0.2211	0.0425	0.0931	0.6883**
LFC	G	-1.7969	1.851	1.4498	-0.5704	-0.6416	-0.265	-0.0348	-0.0709	0.58	0.1157	0.0062	-0.1108	-0.1601	0.0578	0.0889	0.499**
TSL	G	-2.4503	2.5562	1.3454	-0.6135	-0.7046	-0.1909	-0.0483	-0.0465	0.7286	0.195	0.0073	-0.1399	-0.2199	0.0941	0.085	0.5977**
BPT	G	-1.3386	1.4559	0.4538	-0.1526	-0.1995	-0.0897	-0.0107	-0.2096	0.3874	0.1356	0.0032	-0.1237	-0.1109	0.0037	-0.0185	0.1858
CLT	G	-2.5559	2.7238	1.1151	-0.5313	-0.6908	-0.1405	-0.0321	-0.0742	1.0943	0.2543	0.0086	-0.2965	-0.2829	0.0645	0.0793	0.7357**
CGT	G	-2.7153	3.0986	0.8588	-0.3868	-0.5303	-0.0836	-0.0257	-0.0776	0.7592	0.3665	0.0093	-0.3744	-0.237	0.0243	0.0502	0.7361**
CWT	G	-2.8094	3.2141	1.1711	-0.4972	-0.6351	-0.1434	-0.0306	-0.0574	0.8184	0.2945	0.0115	-0.3555	-0.2669	0.1049	0.1504	0.9696**
NKR	G	-1.3816	1.9471	0.7233	-0.2738	-0.3904	-0.0467	-0.0107	-0.0413	0.5165	0.2184	0.0065	-0.6282	-0.1331	-0.0102	0.018	0.5137**
KPR	G	-2.5281	2.7164	1.1828	-0.5403	-0.5742	-0.1263	-0.0316	-0.0692	0.9214	0.2584	0.0092	-0.2488	-0.3361	0.0896	0.0656	0.7889**
SHF	G	-0.6779	0.5327	0.4442	-0.1909	-0.1185	-0.0489	-0.0145	-0.0025	0.2254	0.0284	0.0039	0.0204	-0.0962	0.3131	0.0263	0.4451**
HGW	G	-1.6781	1.6708	0.7642	-0.3358	-0.3238	-0.0938	-0.0163	0.0155	0.3457	0.0733	0.0069	-0.0449	-0.0879	0.0328	0.251	0.5795**

3.3. Deep profiling of agronomic performance and heterosis

Evaluated by per se performance and GCA, the elite line N14 proved to be the paramount general combiner. It exhibited highly significant positive GCA effects for single plant yield (23.81), cob weight (25.84), 100-grain weight (6.55), and shelling percentage (3.02) (Table 2). Considering commercial hybrids CO 6 and NK 6240 as checks, 18 hybrids showed significantly positive heterosis for days to 50% tasselling, ranging from 0.99% to 10.89% over CO 6 and NK 6240 (Table 5). For days to 50% silking, significant positive heterosis ranged from 2.86% to 11.43% over CO 6 and 0.93% to 9.35% over NK 6240. Five hybrids ranged from -29.69% to 15.80%, and fifteen hybrids ranged from -15.87% to 38.56%, showing significant positive heterosis over the check CO 6 and NK 6240, respectively, for plant height. For cob length, the degree of increase of heterosis ranged from -11.15% to 26.63% and -24.6% to 7.47%, with 16 hybrids exhibiting positive heterosis over CO 6 and none of the hybrids expressing positive heterosis over NK 6240, respectively. For cob girth, the increase in heterosis ranged from -10.05% to 10.96% and from -2.65% to 20.09% over CO 6 and NK 6240, respectively, and 7 hybrids for CO 6 and 13 hybrids for NK 6240 exhibited positive

heterosis. The degree of increase in cob weight ranged from -43.43% to 19.83% and from -27.88% to 52.79% over CO 6 and NK 6240, respectively; 3 hybrids over CO 6 and 14 hybrids over NK 6240 exhibited positive heterosis. For shelling percentage, 6 hybrids each exhibited positive heterosis. The heterotic value ranged from -16.47% to 6.5% over CO 6 and -15.936% to 7.19% over NK 6240. For 100-grain weight, none of the hybrids showed positive heterosis over CO 6, but it showed negative heterosis ranging from -35.3% to -1.12% over CO 6. These were in accordance with the results of (Immanuel *et al.*, 2006; Devi *et al.*, 2007; Shukla *et al.*, 2016; Singh *et al.*, 2010; Sharma *et al.*, 2017) [10, 6, 27, 28, 24].

When combined with the beta-carotene donor tester UMI 1200-7-26-1-6-1, the resulting hybrid, N14 × UMI 1200-7-26-1-6-1, displayed explosive heterosis *i.e.*, for Absolute Yield with the highest single plant yield across the entire trial at 147.23 g, dramatically outperforming the overall hybrid mean of 95.33 g (Table 6 and 7). In terms of Cob Dimensions, it achieved a massive cob weight of 194.79 g and a shelling percentage of 76.99%. Further, the cross demonstrated an exceptional SCA effect of 33.08 for grain yield, proving exceptional genetic complementation. Finally, the standard heterosis which

indicates translation of biological vigor to commercial viability, N14 × UMI 1200-7-26-1-6-1 cross achieved highly significant standard heterosis for grain yield, registering a 26.73% increase over the benchmark CO 6 check and a massive 51.01% increase

over the NK 6240 check and also the cob features showed the good phenotypic expression of these traits (Figure 2), this was corroborated with the results of (Premalatha, 2006; Talukder *et al.*, 2016 and Ismail *et al.*, 2026) ^[17, 30, 11] over checks.

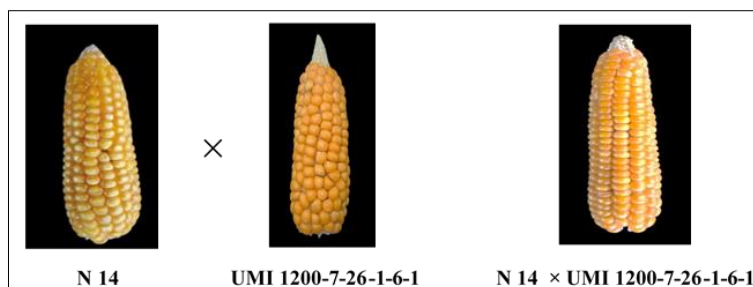


Fig 2: Representation of the promising hybrid N 14 × UMI 1200-7-26-1-6-1 along with its parents

3.4. Trait Associations: Correlation and Path Analysis

To understand the actionable indirect selection criteria, phenotypic and genotypic correlations were scrutinized. Genotypic correlations were consistently higher than phenotypic correlations. Grain yield per plant exhibited highly significant, strong positive genotypic correlations with cob weight (0.9696), number of kernels per row (0.7889), cob length (0.7357), and cob girth (0.7361) (Table 8). Therefore, these were important for improving grain yield per plant. Path coefficient analysis unveiled that Days to 50% Tasselling (3.7246), Plant Height (1.7669), and Cob Length (1.0943) exerted the maximum positive *direct* effects on grain yield (Table 9). Conversely, Days to 50% Silking (-3.9598) exerted a massive *negative* direct effect on yield. The residual effect determines how best the causal factors account for the variability of the dependent factor. Low residual value in this study indicated the adequacy of the characters included in the present study. Thus, to achieve targeting yield in biofortified maize selection for taller plants with extended cob lengths and early silking characteristics will be effective as these are fundamental geometric and temporal drivers of kernel weight which will also reduce the undesirable effect of the component traits studied.

4. Discussion

The comprehensive findings of this study validate the immense technical feasibility and efficiency of MAB in developing elite, nutritionally dense maize. The molecular verification that the favourable 543 bp allele restricts downstream β -carotene conversion thus forcing endosperm accumulation corroborates earlier foundational reports indicating that targeted regulation at the *crtRB1* locus solves PVA deficiency without causing yield penalties (Yan *et al.*, 2010; Babu *et al.*, 2013; Selvi *et al.*, 2014 and Pukalenty *et al.*, 2019) ^[35, 3, 23, 18].

In evaluating combining ability, the overwhelming statistical superiority of SCA variances over GCA variances confirms that yield traits in these diverse populations are primarily governed by non-additive interactions (dominance and epistasis). This strongly aligns with the observations reported by Aminu *et al.* (2014) ^[1], Rajitha *et al.* (2014) ^[20], and Saripalli *et al.* (2017) ^[21]. Crossing geographically and genetically distinct parents (adapted lines with donor testers) forcefully leveraged this variance. The N 14 × UMI 1200-7-26-1-6-1 hybrid capitalized perfectly on this heterosis, vastly out-yielding elite commercial checks. Furthermore, the path analyses identifying cob length and plant height as primary direct drivers of grain yield refine the morphological target for breeders, mirroring conclusions by

Shinde *et al.* (2009) ^[26], Nataraj *et al.* (2014), Kumar *et al.* (2017) and Ismail *et al.* (2026) ^[26, 14, 13, 11].

5. Conclusion

The integration of marker-assisted selection for the *crtRB1* 3'TE allele with conventional Line × Tester breeding is a highly efficient, rapid methodology to generate elite biofortified maize hybrids. Through rigorous biometrical, molecular, and biochemical evaluation, the hybrid N 14 × UMI 1200-7-26-1-6-1 was definitively identified as the paramount genotype. Achieving a massive single plant grain yield of 147.23 g with over 51% standard heterosis, alongside a sharply elevated beta-carotene content of 6.27 μ g/g, this hybrid resolves the conflict between yield drag and nutritional enhancement. In the future, this hybrid can be further advanced through marker assisted backcross breeding to develop high beta carotene maize.

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