

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.604.031>

Molecular Breeding and Variability Analysis of High Beta Carotene Introgressed BC₁F₂ Line of Maize

Pitambar^{1*}, P. Nagarajan² and N. Senthil²

¹Department of Agricultural Biotechnology, Anand Agricultural University,
Anand-388 110, Gujarat, India

²Department of Agricultural Biotechnology, Tamil Nadu Agricultural University,
Coimbatore-641003, India

*Corresponding author

ABSTRACT

Among all the micro nutrient deficiencies, World Health Organization (WHO) had identified Vitamin A deficiency as the more dangerous and widespread micronutrient deficiencies in the world. In order to address this global problem, biofortification of Maize is considered to be a cost effective and sustainable approach. The present study was carried out to raise and identify high β - carotene maize lines of BC₁F₂ (UMI 1200 \times HP 467-15)-C15-S79 population by phenotyping using HPLC along with genotyping of *crtRB1* allele. The randomly selected 10 seeds of selected BC₁F₂ cob were analyzed for β -carotene content through HPLC and the results showed confirmation of improvement in β -carotene content of 3.00 μ g/g respectively. The *crtRB1* allele polymorphism in BC₁F₂ (UMI 1200 \times HP 467-15)-C15-S79 population of the two crosses revealed that the progenies showed segregation distortion (SD) populations, indicating that the favorable allele is under-represented in the population. The variability studies in BC₁F₂ population revealed that, low and moderate GCV and PCV was found for all traits which gives an indication of justifiable variability among the genotypes with respect to these characters and therefore gives scope for improvement of these traits in future generations through selection. The PCV was higher than the corresponding GCV for most of the traits under study which results in high heritability. The heritability estimates of BC₁F₂ generation were found to be highest for plant height. Frequency distribution study of BC₁F₂ population states that for the traits like plant height, ear height and 100 kernel weight, most of the progenies exhibited the values same as or near to recurrent parent. BC₁F₂ population, showed positive skewness for days to 50 per cent tasseling, plant height and ear height while negative skewness was observed for leaf length, days to 50 per cent silking and 100 kernel weight. Negative kurtosis was observed for all the six traits, in BC₁F₂ population. The study indicated that it is possible to develop a high β -carotene inbred with genetic identity closer to that of the recurrent parent in latter generations.

Keywords

Maize, Beta carotene, Variability, Frequency distribution.

Article Info

Accepted:
02 March 2017
Available Online:
10 April 2017

Introduction

Maize (*Zea mays* L.) is the world's most important cereal crop of global importance after wheat. Versatile utility of corn as food and feed enhances the area under production and the productivity day by day (Netravati *et al.*, 2013). Babu *et al.*, (2012) opined that

people in the developed countries also utilize maize for their fast food behaviour. The increasing interest for maize is also due its economic importance which provides industrial raw materials for starch, gluten, corn oil, corn syrup, sugar, corn meal and

corn flour. With all its higher nutritive value maize is deficit in beta carotene (Figure 1). Enriching corn with vitamin A will serve and assure the human population feeding on maize with enriched nutritive factor the solution for major diseases and eye blindness caused by vitamin A deficiency. Markers can be used in the context of Marker-Assisted Back Crossing (MABC) to either control the target gene (foreground selection) or to accelerate the reconstruction of the recurrent parent genotype (background selection). Maize breeders try to combine the nutritional quality traits with yield to produce nutritive inbreds. Selection of favorable gene alleles with inexpensive molecular markers will enable breeders to produce more effectively maize grain with higher provitamin A levels.

Vallabhaneni *et al.*, (2009) and Yan *et al.*, (2010) expressed that the *crtrB1* specifically controls hydroxylation of β -carotene (BC) to β -cryptoxanthin (BCX) in maize endosperm tissues, and its alleles with reduced hydroxylation activity which were associated with increased BC content and BC to BCX ratio (Figure 2). Yan *et al.*, (2010) confirmed that *Zea mays crtrB1* gene is an important gene associated with β -carotene metabolism in maize kernels and identified that three different polymorphisms in *crtrB1* gene *viz.*, 5'TE, InDel4 and 3'TE, are responsible for significant variation of β -carotene concentration in kernels. Owing to the polymorphism of 3'TE (that spans the 6th exon and the 3' UTR) (Yan *et al.*, 2010 and Vignesh *et al.*, 2012), the *crtrB1* gene exists in three allelic states; 3'TE allele 1 (without insertion), 3'TE allele 2 (with 325 bp insertion), and 3'TE allele 3 (with 1250 bp insertion) (Figure 3). The 3'TE allele hierarchy according to kernel β -carotene content is 1>3>2, which means allele 1 is more favourable than the alleles 2 and 3 for higher β -carotene accumulation in kernels and hence allele 1 is termed as favourable allele

and alleles 2 and 3 are termed as unfavourable alleles (Yan *et al.*, 2010).

The success of any crop improvement programme not only dependent on the amount of genetic variability present in the population but also on the extent to which it is heritable, which sets the limit of progress that could be achieved through selection (Wang *et al.*, 2011). Genetic variability for agronomic characters therefore is a key component of breeding programmes for broadening the gene pool of crops (Ahmad *et al.*, 2011). The phenotypic coefficient of variation is the observable variation present in a character or in a population; it includes both genotypic and environmental components of variation and as a result, its magnitude differs under different environmental conditions. The genotypic coefficient of variation on the other hand, is the component of variation which is due to genotypic differences among individuals within a population and is the main concern of plant breeders.

Researchers have reported significant amount of variability in different maize populations including top-crosses and open pollinated varieties (Sampoux *et al.*, 1989). Heritability is a measure of the phenotypic variance attributable to genetic causes and has predictive function in plant breeding. It provides information on the extent to which a particular morphogenetic character could be transmitted to successive generations. Knowledge of heritability influences the choice of selection procedures used by the plant breeder to decide which selection methods would be most useful to improve the character, to predict gain from selection and to determine the relative importance of genetic effects (Laghari *et al.*, 2010).

Keeping the above in mind, the present study deals with following objectives (Figure 4):

Phenotyping of high β -carotene (UMI 1200 \times HP 467-15) BC₁F₂ progenies through HPLC and Identification of favourable *crtRB1* allele in BC₁F₂ population.

Variability and frequency distribution analysis of BC₁F₂ population.

Forwarding selected selfed progenies to generate further BC₁F₃ generation

Materials and Methods

BC₁F₁ population were raised along with the parent UMI 1200 (Pollen source for backcrossing) in summer, 2013. Screening of BC₁F₁ plants and its scoring was done. Selfing of progenies were made to produce BC₁F₂ generation seeds. In the present study BC₁F₂ progenies so produced were analyzed for β -carotene content by HPLC. The selected progenies which were having high β -carotene were raised along with recurrent parents separately in Rabi, 2013 at the Eastern Block, TNAU, Coimbatore with two staggered sowings at 3 and 5 days interval in order to achieve synchronized flowering of parents for hybridization. All the cultural operations were carried out as per the recommendations of crop production guide of Tamil Nadu.

Evaluation of β -carotene content

The randomly selected 10 seeds of selected BC₁F₂ cobs were analyzed for β -carotene content through HPLC for confirmation of improvement in β -carotene content.

Scoring of progenies for *crtRB1* allelic variation

Seedlings were grown in the field for 3 weeks after which fresh leaf tissue of 5-6 seedlings of each inbred line was harvested and stored in Eppendorf tubes at -80°C for total genomic DNA extraction using CTAB method (Dellaporta *et al.*, 1983). The quality and

quantity of DNA was determined by spectrophotometer absorbance at 260 nm. Polymerase chain reaction was performed using *crtRB1* 3'TE gene-specific primers. The segregation pattern of *crtRB1* alleles in all BC₁F₂ segregating progenies were scored in the following pattern. The individuals showing the banding pattern similar to the parent UMI 1200 with the allele size of 296 + 1221 were scored as "AA", the plants with the alleles similar to the parent, HP 467-15 (543 bp allele) were scored as "BB" and the heterozygote's were scored as "AB" (296 + 543 allele size) and tested for their significance using Chi-square test.

Chi-square goodness of fit

The fitness was calculated as below

$$\chi^2 = \sum \frac{(\text{Observed} - \text{expected})^2}{\text{Expected}}$$

The significance is tested by comparing the calculated value and table value and the results were reported as below at 5% level of significance.

Null hypothesis: There is no significant difference between expected ratio and observed ratio

Alternate hypothesis: There is significant difference between expected ratios

Development of BC₁F₃ generation

The BC₁F₂ progenies which were confirmed for high β -carotene content using HPLC and molecular markers were forwarded to develop further generation. The selected BC₁F₂ population was selfed. Selfing was carried out adopting tassel bag method to generate BC₁F₃ which were sown in summer, 2014.

Evaluation of agronomical traits

The back cross progenies were evaluated for the following quantitative characters for their

per se performance. During crop growth, observations on leaf length, days to 50 per cent tasseling and silking were observed.

Before harvest, the tassel length and plant height were recorded. After harvest, 100 kernel weights were recorded. These observations were used for variability analysis.

Variability studies in BC₁F₂ populations

The BC₁F₂ populations were evaluated for 6 quantitative morphological characters. The data was utilized for variability analysis. The various genetic parameters like Phenotypic Coefficients of Variability (PCV), Genotypic Coefficients of Variability GCV, Heritability (h²), Genetic Advance (GA) and Genetic Advance as per cent Mean (GAM), were worked out for the progenies by adopting the formulae given by Johnson *et al.*, (1955).

The phenotypic data of BC₁F₂ progenies along with the parents were utilized for studying the frequency distribution, skewness and kurtosis.

Phenotypic and genotypic variance

The average variance observed in the parent UMI 1200 and HP 467-15 were considered as environmental variance.

The genotypic variance of each generation was estimated by subtracting the estimated environmental variance from the phenotypic variance as follows.

Phenotypic and genotypic coefficients of variability

Based on the calculated Phenotypic and genotypic variance for BC₁F₂ generation PCV and GCV were calculated and scored as in table 1 (Sivasubramanian and Madhava Menon, 1973).

Heritability (h²)

Heritability (h²) estimate in broad sense at five per cent selection intensity were estimated and expressed in percentage.

$$h^2_{\text{(broad sense)}} = \sigma_g^2 / \sigma_p^2 \times 100$$

σ_g^2 = genotypic variance of the population

σ_p^2 = Phenotypic variance of population

The heritability per cent was categorized as below adopted by Robinson *et al.*, (1949).

Heritability in per cent	Category
< 30	Low
31 – 60	Medium
> 60	High

Genetic advance (GA)

Genetic advance was estimated by the method formulated by Johnson *et al.*, (1955).

$$\text{Genetic advance} = k \times h^2 \times \sigma_p$$

Where,

h² = Heritability in broad sense

σ_p = Phenotypic standard deviation

k = Selection differential (at 5 % selection intensity) (*i.e.*) 2.06 (Falconer, 1960)

Genetic advance as per cent of mean (GAM)

The genetic advance as per cent of mean was categorized as suggested by Johnson *et al.*, (1955).

GA as per cent of mean =

$$\frac{\text{Genetic advance}}{\text{Grand mean}} \times 100$$

GA was categorized as:

GA per cent value	Category
< 10 per cent	Low
10 - 20 per cent	Moderate
> 20 per cent	High

Frequency distribution

The phenotypic data of progenies along with the parents were utilized for studying the frequency distribution for leaf length, days to 50 per cent tasseling, days to 50 per cent silking, plant height, ear height and hundred kernel weight. It was calculated by classification into different classes at regular class intervals and the population distribution was obtained pictographically for all the characters under study in which the number of progenies resembling the parental range could be identified.

Skewness and Kurtosis

These were calculated using the frequency distribution of the characters mentioned.

$$\beta_1 = \text{Skewness}$$

If, $\beta_1 > 0$, then positively skewed
 $\beta_1 < 0$, then negatively skewed
 $\beta_1 = 0$, then symmetric distribution

$$\beta_2 = \text{Kurtosis}$$

If, $\beta_2 > 1$, then leptokurtic
 $\beta_2 < 1$, then platykurtic
 $\beta_2 = 0$, then mesokurtic

$$\beta_1 = \frac{\mu_3}{\mu_2^{3/2}}$$

$$\beta_2 = \frac{\mu_4}{\mu_2^2}$$

Where,

$$\mu_2 = \frac{1}{N} \sum f_i (x_i - \bar{x})^2$$

$$\mu_3 = \frac{1}{N} \sum f_i (x_i - \bar{x})^3$$

$$\mu_4 = \frac{1}{N} \sum f_i (x_i - \bar{x})^4$$

Where,

X_i is the individual observation

\bar{X} is the mean of the character under observation and

N is the number of observations

Statistical analysis

For the quantitative morphological characters descriptive statistics were carried out using XLSTAT software to estimate mean, range, standard deviation and variance. The Chi-square goodness of fit was estimated using XLSTAT software. The phenotypic data of BC_2F_1 and BC_1F_2 progenies were subjected for variability analysis using XLSTAT software and frequency distribution, skewness and kurtosis by SPSS.16.

Results and Discussion

The present investigation was carried out to develop high β -carotene maize by introgressing *cr1RBI* allele through marker assisted backcross breeding. BC_1F_2 progenies were analyzed for β -carotene content by HPLC. The selected progenies identified to contain high β -carotene by HPLC profiling were raised and genotyping of *cr1RBI* allele was done. Based on marker data analysis plants having favourable allele 1(543bp) were selected for selfing to develop BC_1F_3 generation seeds.

Estimation of β -carotene content BC_1F_2 progenies

The randomly selected 10 seeds of selected BC_1F_2 (Plate 1) cobs were analyzed for β -carotene content through HPLC for confirmation of improvement in β -carotene content. The results revealed that β -carotene content of BC_1F_2 (UMI 1200 \times HP 467-15)-

C15-S79) was 3.00µg/g (Figure 8) and the progeny was forwarded to develop BC₁F₂ population.

Foreground marker screening in (UMI 1200 × HP467-15)-C15-S79 BC₁F₂ population

Among the 100 plants of (UMI 1200 × HP467-15)-C15-S79 BC₁F₂ population, 52 progenies showed both parent’s alleles ‘AB’ (Heterozygotes with allele size of 296+543bp), 18 progenies showed ‘B’ alleles (Allele size of 543 bp) and 30 progenies showed ‘A’ alleles (Allele size of 296 bp). Progenies showing homozygous to favourable allele1 (546bp) viz., ‘BB’ is selected for selfing to develop BC₁F₃ generation seeds (Figure 5).

Chi -square goodness of fit

The Chi-square test for goodness of fit was calculated for allele frequency (Table 2). The progenies showed deviation in expected segregating ratio (1: 2: 1) with respect the crtRB1 gene specific marker. In F₂ population, segregation disorder (SD) was skewed towards the unfavourable homozygous and/or heterozygote allele and the favourable allele was under-represented in the BC₁F₂ population.

The Chi-Square test results revealed that the progenies showed deviation in expected

segregating ratio of BC₁ F₂ (1: 2:1) populations of the cross UMI 1200 × HP467-15 with respect the crtRB1 gene specific marker, where segregation disorder(SD) was observed for crtRB1 in BC₁F₂ populations. In BC₁F₂ population, segregation disorder (SD) was skewed toward the unfavourable homozygous allele and the favorable allele was under-represented. The present results were in confirmation with the earlier reports. Lu *et al.*, (2002) opined that the observation of segregation disorder (SD) for *LcyE* in all eight and for *crtRBI* in five of the eight digenic F₂ populations was consistent with frequent observation of SD in maize and presence of many segregation distortion regions (SDRs) throughout the maize genome. Segregation distortion (SD) in maize could be due to the presence of gametophytic factors (ga) (Mangelsdorf and Jones 1926; Neuffer *et al.*, 1997) or to naturally occurring gene mutants like dek (defective kernel), ms (male sterile) and emb (embryo-specific mutation) (Neuffer *et al.*, 1997).

Development of BC₁F₃ progenies

The individuals possessing the crtRBI loci in homozygous condition (BB) were selfed to produce BC₁F₃ generation (UMI 1200 × HP 467-15)-C15-S79-S7). The β-carotene content of BC₁F₃ progenies so produced will be estimated and then raised during Summer, 2014.

Table.1 PCV and GCV of BC₁F₂

Phenotypic Coefficient of Variation (%) (PCV)	$\frac{\sqrt{\text{Phenotypic variance}}}{\text{Grand mean}} \times 100$	
Genotypic Coefficient of Variation (%) (GCV)	$\frac{\sqrt{\text{Genotypic variance}}}{\text{Grand mean}} \times 100$	
Scoring for PCV and GCV variability	PCV and GCV	Category
	< 10 per cent	Low
	10 – 20 per cent	Moderate
	> 20 per cent	High

Table.2 Chi-square goodness of fit for BC₁F₂ segregating population of (UMI 1200 × HP467-15)-C15-S79

Population	Marker	Total No. of plants	Observed values			χ^2
			Heterozygous (AB)	Homozygous (AA)	Homozygous (BB)	
UMI 1200 x HP467-15	crtRB1	100	52	30	18	27.04

The table value of χ^2 (df =1) at 5% level is 5.91. The calculated value is more than calculated value. There is deviation between expected ratio and observed ratio.

Table.3 Descriptive statistics of BC₁F₂ segregating generation (UMI 1200 × HP 467-15)-S15-S79

Parameters	Parental lines		Mean	Range	Standard deviation	Variance
	UMI 1200	HP467-15				
Leaf length (cm)	51.6	42.3	53.54	40-59	5.35	28.64
50 % tasseling (days)	60	56	59	54-64	3.6	12.99
50% silking (days)	63	59	61	57-64	2.69	7.42
Plant height (cm)	128	108.8	132.61	105-138	6.59	44.5
Ear height (cm)	64.2	45	69	44-74	5.6	31
100 kernel weight (g)	26	22.9	28.2	20-30	2.48	6.15

Table.4 Variability analysis in BC₁F₂ population of (UMI 1200 × HP 467-15)-S15-S79

Parameters	PCV %	GCV %	h ² %	GA	GAM
Leaf length (cm)	11.73	10.2	64.40	7.8	15.5
50 % tasseling (days)	4.40	4.36	54.3	5.01	8.88
50% silking (days)	3.2	2.42	55.53	2.20	3.72
Plant height (cm)	6.03	5.93	97.05	14.75	12.04
Ear height (cm)	11.91	4.30	66.07	1.91	3.21
100 kernel weight (g)	8.38	7.2	71.8	0.34	1.2

GCV, PCV and GA as mean: Low - < 10 %; Moderate 10 – 20%; High > 20%

h²: Low - < 30 %; Medium- 31 – 60 %; High - > 60 %

(PCV –Phenotypic coefficient of variability, GCV- Genotypic coefficient of variability, h² –Heritability, GA- Genetic advance and GAM - Genetic advance as per cent mean)

Table.5 Skewness and kurtosis variation in BC₁F₂ Population
(UMI 1200 × HP 467-15)-S15-S79

Traits	Skewness	Kurtosis
	BC ₁ F ₂	BC ₁ F ₂
Leaf length (cm)	-0.04	-1.32
50 % tasseling (days)	0.41	-0.6
50% silking (days)	-0.12	-0.75
Plant height (cm)	0.06	-1.44
Ear height (cm)	0.04	-0.80
100 kernel weight (g)	-0.026	-1.05

(<0) - Negatively skewed; (> 0) - Positively skewed; (0) – Normal distribution

Table.6 HPLC analysis of β-carotene content of parents (UMI 1200 and HP467-15) and selected BC₁F₂ progeny

Parents/progenies	Pedigree	β-carotene(μg/g)
Recurrent	UMI1200	1.16
Donor	HP467-15	5.1
BC ₁ F ₂	(UMI1200×HP467-15)-C15-S79	3.00

Plate.1 Variability in cob characters



UMI 1200 (Recurrent parent)



HP467-15 (Donor parent)



BC1F2

[Pedigree: (UMI1200×HP467-15)-C15-S79]



BC1F3

[Pedigree: (UMI1200×HP467-15)-C15-S79-S7]

Figure.1 Structure of β -carotene

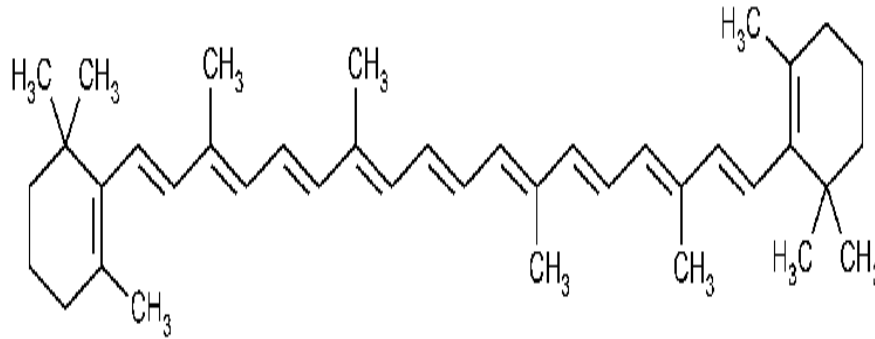


Figure.2 Carotenoid biosynthetic pathway in plants

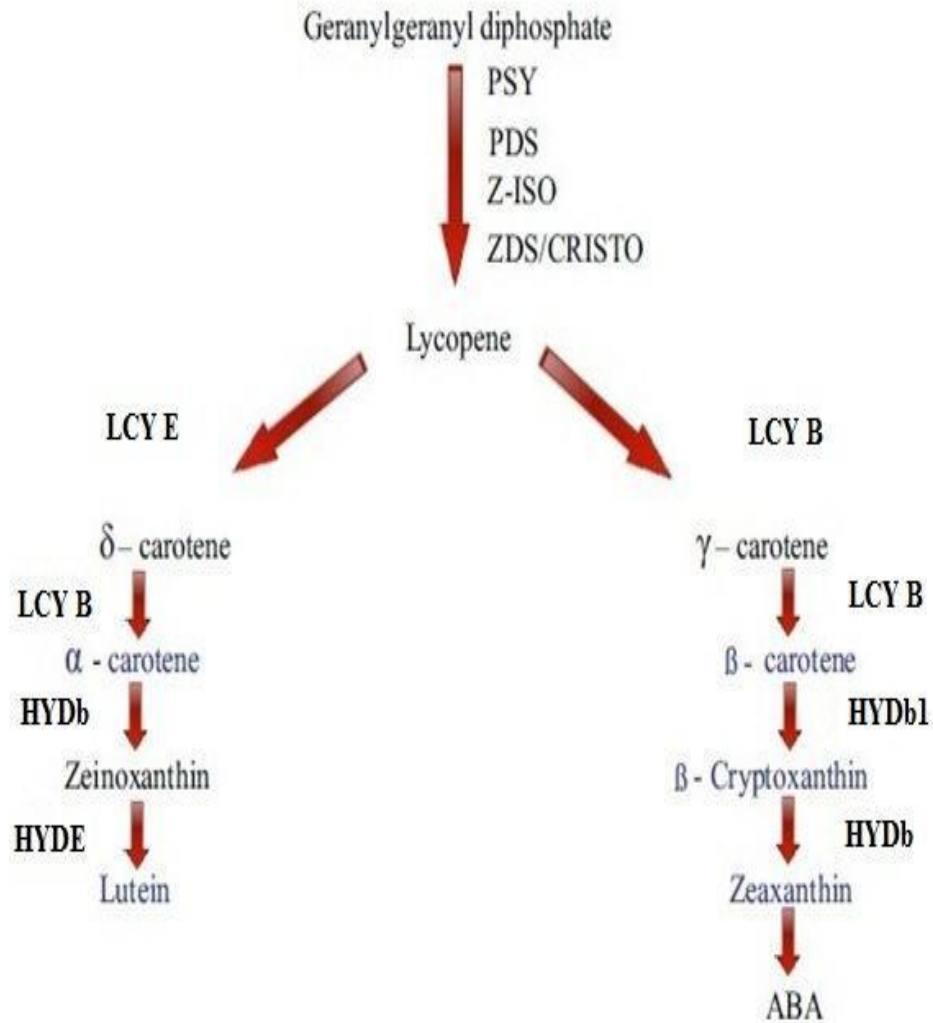


Figure.3 Genetic variation at *crtRB1* 3' TE (Modified from Yan *et al.*, 2010). The genetic variation due to insertions at "3' TE" (a polymorphic site of the *crtRB1* gene results three alleles that are associated with variation in β -carotene accumulation in the kernel. Allele 1 is without insertion and a PCR of this allele with the indicated primer set results in an amplicon of size 543bp. Allele 2 has a 325bp insertion and a PCR of this allele with the indicated primer sets results in an amplicon of size 296 + 875bp. Allele 3 has a 1250bp insertion and a PCR of this allele with the indicated primer sets results in an amplicon of size 296+1221+1800 (However, the largest amplicon (1800 bp) amplified by the primers 65F and 66R was usually weak or not amplified). Of the three alleles of 3' TE, allele 1 is known as a favorable allele, for it is associated with the enhancement of β -carotene concentration in the maize grain.

Three 3' TE alleles of *crtRB1* gene

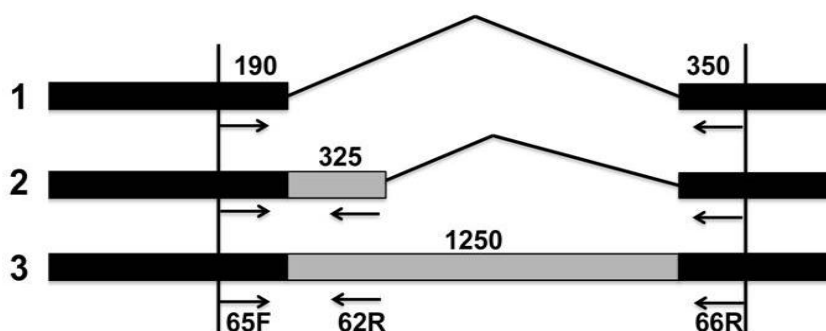


Figure.4 Breeding programme for introgression of *crtRB1* allele into UMI 1200 by marker assisted selection. The populations used (*i.e.*, P1 and P2) or developed (*i.e.*, F1 and BC1F1) in the previous study are shown in black fonts and the populations used (*i.e.*, BC1F2) or developed (*i.e.*, BC1F3,) in the present study are shown in red fonts

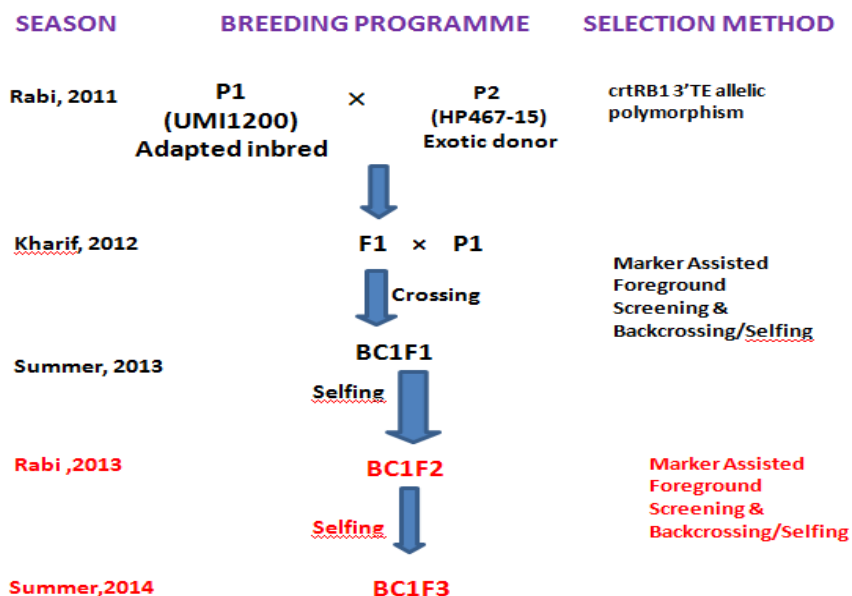


Figure.5 Screening of foreground marker (*crtRBI* 3'TE alleles) in BC1F2 population [pedigree: (UMI1200×HP467-15)-S15-S79] L denotes the marker lane. P1 and P2 denote the parents UMI 1200 and HP467-15 respectively. The numbers above the lanes refer to the progenies analyzed in BC1F2 population (numbers in red are selected progenies selfed)

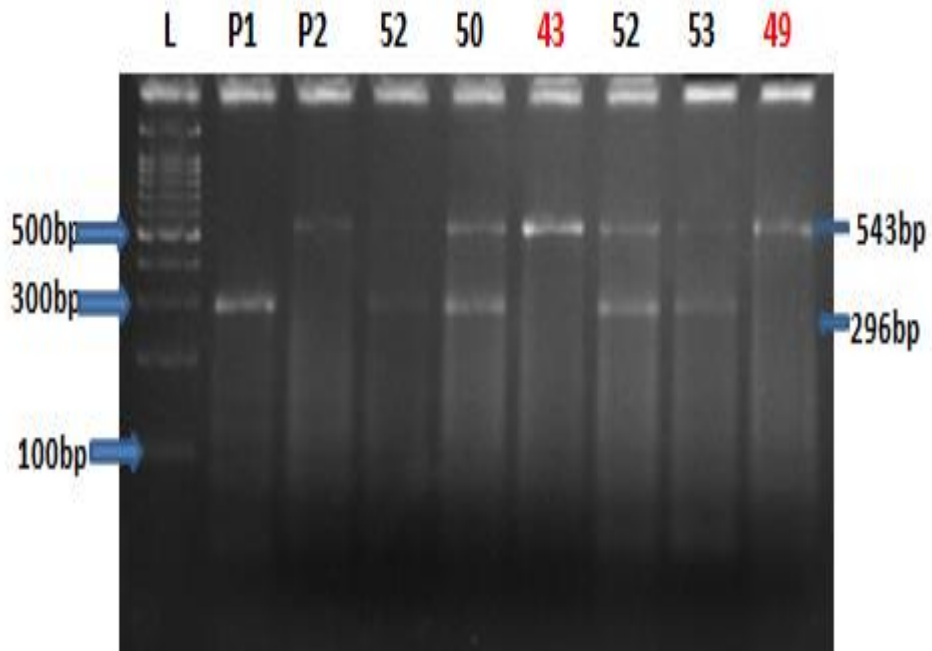
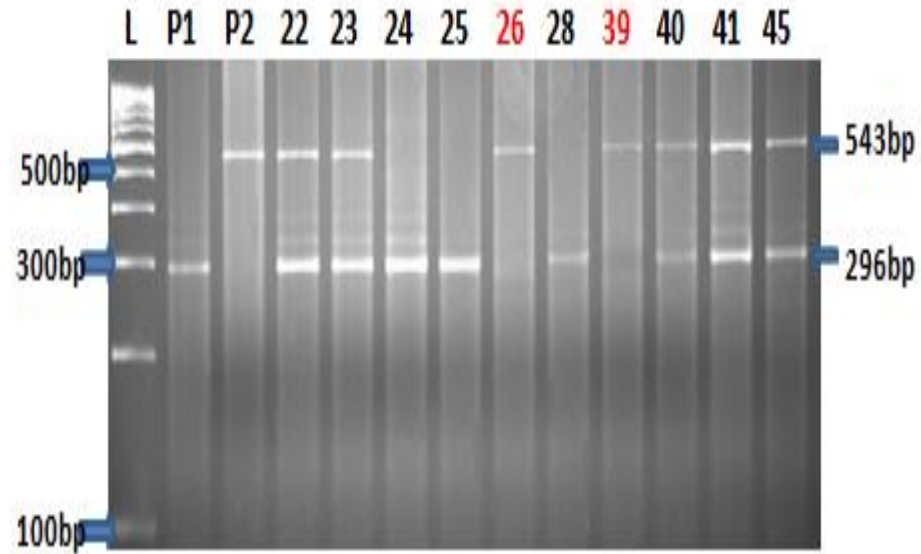


Figure.6 Frequency distribution curve for morphological characters of BC₁F₂ population [Pedigree: (UMI 1200 × HP 467-15)-S15-S79]

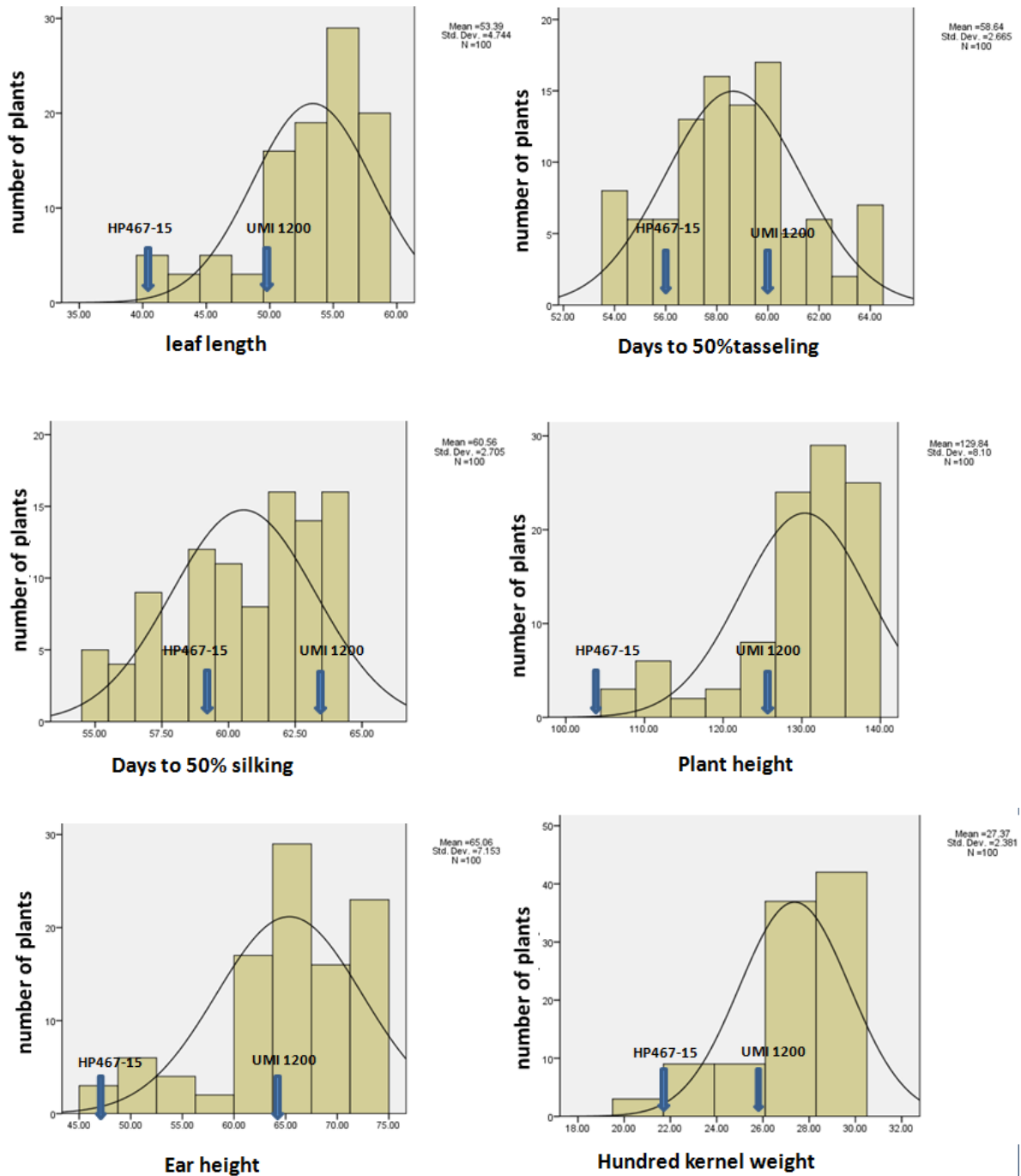


Figure.7 HPLC Chromatogram for β -carotene content of Donor (HP467-15) and Recurrent (UMI1200) parents

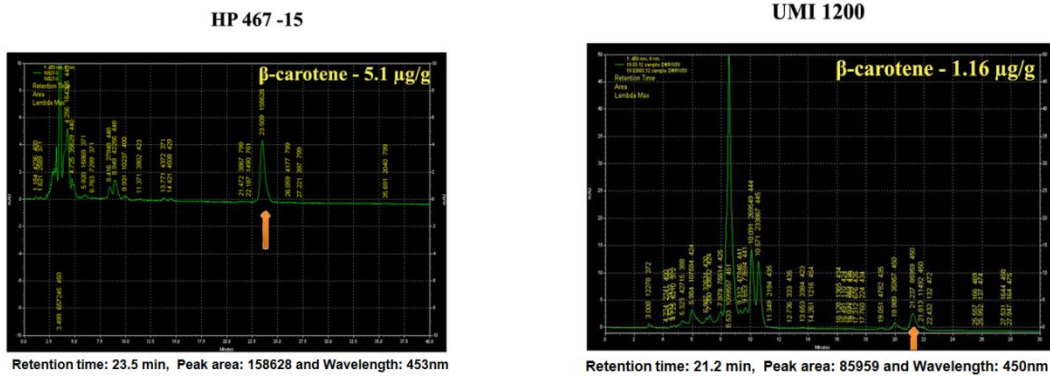
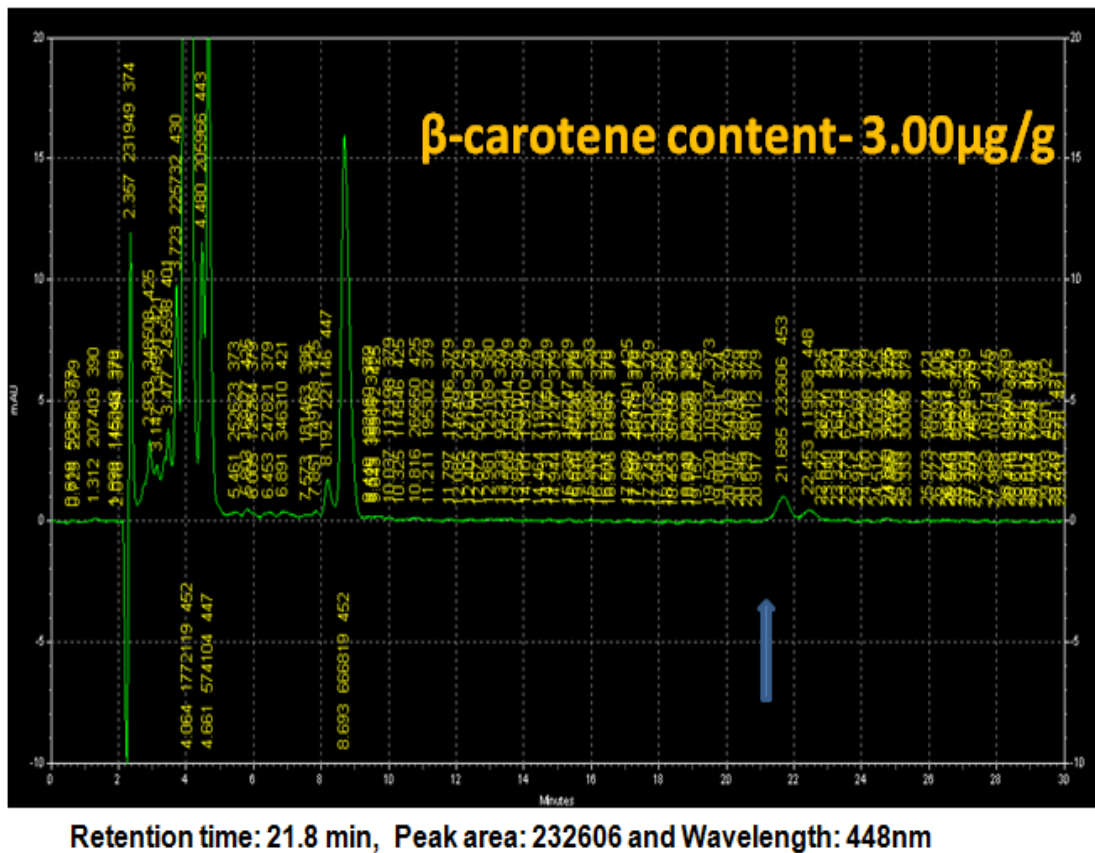


Figure.8 HPLC Chromatogram for β -carotene content of BC₁F₂ progeny [Pedigree: UMI 1200×HP467-15)-C15-S79]



Performance of BC₁F₂ generation of (UMI 1200 × HP 467-15)-S15-S79

The BC₁F₂ population (UMI 1200 × HP 467-15)-S15-S79 progeny showed higher, range of

variability for the traits viz., plant height (105-138cm) with mean value of 132.4cm indicating the segregation of BC₁F₂ progeny, while the days to 50 per cent tasseling ranged from 54-62 days with a mean value of 59

days. Days to 50 per cent silking ranged from 57-64 days with a mean value of 61 days. The BC₁F₂ progeny (UMI 1200 × HP 467-15)-S15-S79 from the cross displayed maximum variance of 44.5 and 31 for plant height and ear height respectively (Table 3).

Variability studies in (UMI 1200 × HP 467-15)-S15-S79 BC₁F₂ segregating generation

The values of various variability parameters pertaining to BC₁F₂ segregating populations of the cross (UMI 1200 × HP 467-15)-S15-S79 were presented (Table 4).

The percentage of PCV was less than 10 per cent (low variability) for days to 50 per cent tasseling (4.40%), days to 50 per cent silking (3.2%), plant height (6.03%) and hundred kernel weight (8.38%). PCV exhibited a moderate variability of 10-20 per cent for leaf length (11.73%) and ear height (11.91%). The percentage of GCV was less than 10 per cent (low variability) for days to 50 per cent tasseling (4.36%), days to 50 per cent silking (2.42%), plant height (5.93%), ear height (4.3%) and hundred kernel weight (7.2%) while genotypic coefficient of variation exhibited a moderate variability (10- 20 %) for leaf length (10.2%).

These characters had moderate GCV and PCV which gives an indication of justifiable variability among the genotypes with respect to these characters and therefore gives scope for improvement of these traits in future generations through selection. The minor variation between values of GCV and PCV shows the limited role of environment in these characters and the heritability was very high for these traits. Similar results were reported by Bello *et al.*, (2012).

The heritability estimates of BC₁F₂ generation of the (UMI 1200 × HP 467-15)-S15-S79 cross were found with highest heritability for plant height (97.05%) while high heritability

was found for hundred kernel weight (71.8%), ear height (66.07%) and leaf length (64.4%). Days to 50% tasseling and silking was found to have medium heritability. Characters with high heritability could easily be fixed with simple selection resulting in quick progress. However, it had been accentuated that heritability alone had no practical importance without genetic advance (Najeeb *et al.*, 2009).

Among the traits, highest genetic advance was exhibited for plant height (14.75). All other traits were found to have low genetic advance. High genetic advance coupled with high heritability estimates offers the most suitable condition for selection. This is the indication of predominance of additive gene action. This is desirable for selection since these are least influenced by the environment. Researchers (Rafique *et al.*, 2004; Akbar *et al.*, 2008; Rafiq *et al.*, 2010) had reported that high heritability and high genetic advance for different yield controlling traits in maize. Therefore, availability of good knowledge of these genetic parameters existing in different yield contributing characters and the relative proportion of this genetic information in various quantitative traits is a pre-requisite for effective crop improvement.

The medium genetic advance as per cent of mean of 10-20 per cent was observed for plant height (12.04%) and leaf length (15.5%) while low genetic advance as per cent of mean of <10 per cent was observed for all other traits.

Frequency distribution of (UMI 1200 × HP 467-15)-S15-S79 BC₁F₂ population

In (UMI 1200 × HP 467-15)-S15-S79 cross BC₁F₂ population (Figure 6), for the traits like plant height, ear height and 100 kernel weight, most of the progenies exhibited the values same as recurrent parent. For leaf length most of the progenies were found to

possess values more than either parents. When traits like days to 50 per cent tasseling, days to 50 per cent silking are considered most of the progenies found to possess values less than either of parents.

Positive skewness was observed for traits like days to 50% tasseling, plant height and ear height. All other traits showed negative skewness (Table 5). The positive skewness indicates the presence of complementary epistatic gene action for the trait and the gain is slower with mild selection and gain is faster with intensive selection. The negative skewness indicates the presence of duplicate epistasis gene action and the gain is faster with mild selection and rapid with intense selection (Snape and Riggs, 1975).

Negative kurtosis (platykurtic curve) was observed for all the traits. The negative kurtosis indicate platykurtic curve which means that flat values are present in the distribution and complementary gene action. If selection for these characters were made intensively, the gain will be faster (Table 5).

Improvement in β -carotene content by introgression of favourable *crtRB1* allele from exotic donor line (HP467-15) into popular inbred (UMI 1200) through marker assisted backcrossing

Both Recurrent (UMI 1200) and Donor (HP467-15) parents were analyzed for β -carotene content through HPLC and the results showed that UMI 1200 (Recurrent parent) has β -carotene content of 1.16 $\mu\text{g/g}$ and HP467-15 (Donor parent) has β -carotene content of 5.1 $\mu\text{g/g}$ (Figure 7). BC_1F_2 showed confirmation of improvement in β -carotene content of 3.00 $\mu\text{g/g}$ (UMI1200 \times HP467-15)-C15-S79 (Figure 8). These were identified as promising bio-fortified lines and utilized for later generations (Table 6).

Thus the study of the evaluated populations indicated that it is possible to develop a high β -carotene inbred with genetic identity closer to that of the recurrent parent in latter generations.

In conclusion the present study was carried out to raise and identify high β -carotene lines of BC_1F_2 population by phenotyping using HPLC along with genotyping of *crtRB1* allele. As per the continuation of previous work the BC_2F_1 and BC_1F_2 seeds were collected by the ongoing GOI-DBT project "Marker assisted introgression of *LycE* gene for enhanced ProA in maize". The seeds of BC_1F_2 progenies identified to contain high β -carotene by HPLC were raised to generate BC_1F_2 population and field studies were carried. The *crtRB1* allele specific foreground marker was used for identifying 543bp favorable allele in the segregating populations under evaluation.

The randomly selected 10 seeds of each selected BC_1F_2 progenies were analyzed for β -carotene content through HPLC and the results showed confirmation of improvement in β -carotene content of 3.00 $\mu\text{g/g}$ (*i.e.*, up to 3 fold increase over the parent) and were identified as promising bio-fortified lines and utilized for later generations. The seeds of BC_1F_2 progenies identified to contain high β -carotene by HPLC were raised to generate BC_1F_2 (UMI 1200 \times HP 467-15)-C15-S79 population of 100 progenies out of which, 18 progenies were identified to be homozygous for favourable allele1 (546 bp). The *crtRB1* allele polymorphism in BC_1F_2 population of the cross revealed that the progenies showed segregation distortion (SD) *i.e.*, deviation from the expected segregating ratio BC_1F_2 (1:2:1) populations, indicating that the favourable allele is under-represented in the population. The variability studies BC_1F_2 populations of the crosses revealed that, the BC_1F_2 population of (UMI 1200 \times HP 467-

15)-C15-S79, the percentage of PCV was less than 10 per cent (low variability) for days to 50 per cent tasseling (4.40%), days to 50 per cent silking (3.2%), plant height (6.03%) and hundred kernel weight (8.38%) The percentage of GCV was less than 10 per cent (low variability) for days to 50 per cent tasseling (4.36%), days to 50 per cent silking (2.42%), plant height (5.93%), ear height (4.3%) and hundred kernel weight (7.2%). The heritability estimates of BC₁F₂ generation of the (UMI 1200 × HP 467-15)-C15-S79 cross were found with highest heritability for plant height (97.05%) while high heritability was found for hundred kernel weight (71.8%), ear height (66.07%) and leaf length (64.4%). Days to 50% tasseling and silking was found to have medium heritability. Among the traits, highest genetic advance was exhibited for plant height (14.75). All other traits were found to have low genetic advance. The medium genetic advance as per cent of mean of 10–20 per cent was observed for plant height (12.04%) and leaf length (15.5%) while low genetic advance as per cent of mean of <10 per cent was observed for all other traits

Frequency distribution study of BC₁F₂ population states that for the traits like plant height, ear height, 100 kernel weight, most of the progenies exhibited the values same as or near to recurrent parent. The skewness obtained from the frequency distribution of the present study revealed that for (UMI 1200 × HP 467-15)-C15-S79 BC₁F₂ population, positive skewness was observed for traits like days to 50% tasseling, plant height and ear height. All other traits showed negative skewness. Negative kurtosis (platykurtic curve) was observed for all the six traits, in both BC₂F₁ and BC₁F₂ population.

Thus the variability studies of the evaluated populations indicated that it is possible to develop a high β-carotene inbred with genetic

identity closer to that of the recurrent parent in latter generations. The study also revealed that information about the extent of variation, estimates of heritability and expected genetic advance in respect of maize grain yield and yield contributing characters constitutes the basic requirement for a crop improvement programme.

References

- Ahmad, S.Q., S. Khan, M. Ghaffar and F. Ahmad. 2011. Genetic diversity analysis for yield and other parameters in maize (*Zea mays* L.) genotypes. *Asian J. Agric. Sci.*, 3(5): 385-388
- Akbar, M., M.S. Shakoor, A. Hussain and M. Sarwar. 2008. Evaluation of maize 3-way crosses through genetic variability, broad sense heritability, characters association and path analysis. *J. Agric. Res.*, 46(1): 39-45.
- Babu, R., Rojas, N.P., Gao, S., Yan, J. and Pixley, K. 2012a. Validation of the effects of molecular marker polymorphisms in *LcyE* and *CrtRB1* on provitamin A concentrations for 26 tropical maize populations. *Theor. Appl. Gene.*, DOI 10.1007/s00122-012-1987-3.
- Bello, O.B., S.A. Ige, M.A. Azeez, M.S. Afolabi, S.Y. Abdulmalik and J. Mahamood. 2012. Heritability and Genetic Advance for Grain Yield and its 140 Component Characters in Maize (*Zea Mays* L.). *Intl J. Pl. Res.*, 2(5): 138-145.
- Dellaporta, S.L., J. Wood and J.B. Hicks. 1983. A plant DNA Mini preparation version 2. *Plant Mol. Biol. Rep.*, 1: 19- 22.
- Falconer, D.S. 1960. Introduction to Quantitative Genetics. Oliver and Boyd, Edinburgh. 340.
- Johnson, D. and W. Russell. 1982. Genetic variability and relationships of physical grain-quality traits in the BSSS population of maize. *Crop Sci.*, 22: 805-809.
- Johnson, H.W., J.F. Robinson and R.E. Comstock. 1955. Estimates of genetic and environmental variability in soybean. *Agron. J.*, 47: 314 - 318.
- Laghari, K.A., M.A. Sial, M.A. A. Arain, A.A. Mirbahar, A.J. Pirzada, M.U. Dahot and

- S.M. Mangrio. 2010. Heritability studies of yield and yield associated traits in bread wheat. *Pak. J. Bot.*, 42(1): 111-115.
- Lu, H., J. Romero-Severson and R. Bernardo. 2002. Chromosomal regions associated with segregation distortion in maize. *Theor. Appl. Genet.*, 105: 622–628.
- Mangelsdorf, P.C. and D.F. Jones. 1926. The expression of Mendelian factors in the gametophyte of maize. *Genetics*, 11: 423–455.
- Najeeb, S., A.G. Rather, G.A. Parray, F.A. Sheikh and S.M. Razvi. 2009. Studies on genetic variability, genotypic correlation and path coefficient analysis in maize under high altitude temperate ecology of Kashmir. *Maize Genetics Cooperation Newslett.*, 83: 1-8.
- Netravati, G. Shantakumar, A. Sateesh, M. Laxman and G. Prakash. 2013. Heterosis Breeding for Maturity, Yield and Quality Characters in Maize (*Zea mays* L.). *Mol. Pl. Breeding*, 4(6): 44-49.
- Neuffer, M.G., E.H. Coe and S.R. Wessler. 1997. Mutants of maize. Cold Spring Harbor Laboratory Press, USA
- Rafiq, C.M., M. Rafique, A. Hussain and M. Altaf. 2010. Studies on heritability, correlation and path analysis in maize (*Zea mays* L.). *J. Agric. Res.*, 48(1): 35-38.
- Rafique, M., A. Hussain, T. Mahmood, A.W. Alvi and B. Alvi. 2004. Heritability and interrelationships among grain yield and yield components in maize (*Zea mays* L.). *Int. J. Agri. Biol.*, 6(6): 1113-1114.
- Robinson, H.F., R.E. Comstock and P.H. Harvey. 1949. Estimates of heritability and the degree of dominance in corn. *Agron. J.*, 41: 353 - 359.
- Sampoux, J.P., A. Gallais and M. Lefort-Buson. 1989. S1 value combined with topcross value for forage maize selection. *Agronomie*, 9: 5, 511-520.
- Sivasubramanian, S. and P. Madhava Menon. 1973. Genotypic and phenotypic variability in rice. *Madras Agric. J.*, 60: 1093 - 1096.
- Snape, J.W and T.S. Riggs. 1975. Genetical consequences of single seed descent in the breeding of self pollinated crops. *Heredity*, 35: 211 - 219.
- Vallabhaneni, R., C.E. Gallagher, N. Licciardello, A.J. Cuttriss, R.F., Quinlan and E.T. Wurtzel. 2009. Metabolite sorting of a germplasm collection reveals the hydroxylase3 locus as a new target for maize provitamin A biofortification. *Plant Physiol.*, 151: 1635-1645.
- Vignesh, M., Firoz Hossain, T. Nepolean, Supradip Saha, P.K. Agrawal, S.K. Guleria, B.M. Prasanna and H.S. Gupta. 2012. Genetic variability for kernel β -carotene and utilization of crtRB1 3'TE gene for biofortification in maize (*Zea mays* L.). *Indian J. Genet.*, 72(2): 189-194.
- Wang, X., Chang, J., Qin, G., Zhang, S., Cheng, X. and C. Li. 2011. Analysis on yield components of elite maize variety Xundan 20 with super high yield potential. *Afr. J. Agric. Res.*, 6(24): 5490-5495.
- Yan, J.B., C.B. Kandianis, C.E. Harjes, L. Bai, E.H. Kim, X.H. Yang, D.J. Skinner, Z.Y. Fu, S. Mitchell, Q. Li, M.G. Fernandez, M. Zaharieva, R. Babu, Y. Fu, N. Palacios, J.S. Li, D. DellaPenna, T. Brutnell, E.S. Buckler, M.L. Warburton and T. Rocheford. 2010. Rare genetic variation at *Zea mays crtRB1* increases β -carotene in maize grain. *Nat. Genet.*, 42: 322–327.

How to cite this article:

Pitambar, P. Nagaraja and Senthil, N. 2017. Molecular Breeding and Variability Analysis of High Beta Carotene Introgressed BC₁F₂ Line of Maize. *Int.J.Curr.Microbiol.App.Sci.* 6(4): 270-287. doi: <https://doi.org/10.20546/ijemas.2017.604.031>

Annexure-I

Foreground marker (crtRB1 3'TE) allelic polymorphism of (UMI 1200 × HP467-15)-C15-S79 BC₁F₂ population

Progeny No.	Allele size (bp)	Progeny No.	Allele size (bp)	Progeny No.	Allele size (bp)
1	296	43	543	85	296+543
2	543	44	296	86	296
3	296	45	296+543	87	296
4	296+543	46	296	88	296
5	543	47	543	89	543
6	296	48	296+543	90	296
7	543	49	543	91	296
8	296+543	50	296+543	92	296+543
9	543	51	296	93	296
10	296+543	52	296+543	94	296
11	296	53	296+543	95	296
12	543	54	296+543	96	296+543
13	296	55	296	97	296+543
14	296+543	56	296+543	98	296+543
15	296	57	296	99	296
16	543	58	296	100	296
17	296+543	59	296		
18	296	60	296+543		
19	296+543	61	296+543		
20	543	62	296		
21	296+543	63	296		
22	296+543	64	296+543		
23	296+543	65	296+543		
24	296	66	543		
25	296	67	543		
26	543	68	296+543		
27	296+543	69	296		
28	296	70	296+543		
29	296+543	71	296+543		
30	296	72	296		
31	296	73	296+543		
32	296	74	543		
33	296+543	75	296+543		
34	296	76	296		
35	296	77	296+543		
36	296	78	296		
37	296	79	543		
38	543	80	296		
39	543	81	296		
40	296+543	82	296+543		
41	296+543	83	296		
42	296	84	296 +543		
Allele type 'AA' - 296 bp; Allele type 'BB'- 543 bp; Allele type 'HH'- 296 + 543 bp					